ON THE DEVELOPMENT OF DIABETES MELLITUS IN CHINESE HAMSTERS GIVEN STREPTOZOTOCIN AND N-NITROSOMETHYLUREA

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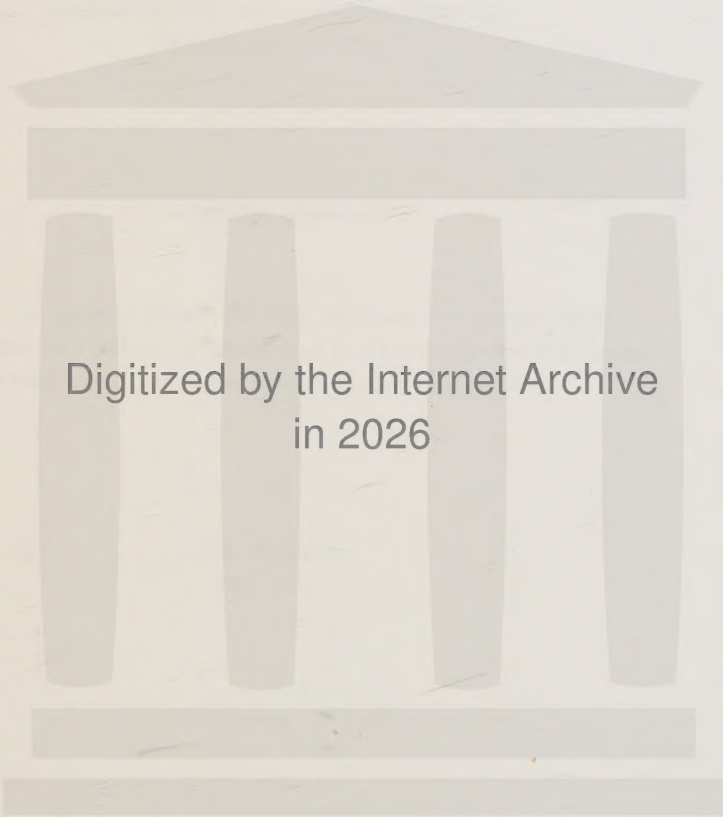
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ON THE DEVELOPMENT OF DIABETES MELLITUS IN CHINESE HAMSTERS GIVEN STREPTOZOTOCIN AND N-NITROSOMETHYLUREA

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This thesis is based upon the following publications, as well as some new results.

I Wilander, E., and Boquist, L.: Streptozotocin-diabetes in the Chinese hamster. Blood glucose and structural changes during the first 24 hours. Horm. Metab. Res. 4:426-433, 1972.

II Wilander, E.: Streptozotocin-diabetes in the Chinese hamster. Volumetric quantitation of the pancreatic islets and inhibition of diabetes with nicotinamide. In manuscript 1974.

III Wilander, E.: Streptozotocin-diabetes in the Chinese hamster. Long-term effects on the light microscopic morphology of the pancreatic islet tissue, liver and kidney. In manuscript 1974.

IV Wilander, E., and Gunnarsson, R.: Diabetogenic effects of N-nitrosomethylurea in the Chinese hamster. In manuscript 1974.

V Wilander, E.: An ultrastructural study of pancreatic islet cell destruction by N-nitrosomethylurea. In manuscript 1974.

VI Wilander, E., and Tjälve, H.: On the ability of N-nitrosomethylurea to be diabetogenic by accumulating in the pancreatic islets. In manuscript 1974.

These papers will be referred to in the following text by their Roman numerals.

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INTRODUCTION

The relatively recent report by Tattersall and Pyke (1972) that the hereditary trait for human juvenile diabetes mellitus is not very strong has increased the interest in environmental factors in the pathogenesis of this type of diabetes.

From the point of view of a pathologist an important finding by Maclean and Ogilvie (1955 and 1959), and later by Gepts (1965), is the atrophy of the pancreatic islets occurring in human juvenile diabetes mellitus. They have also found that the atrophy is more pronounced in people with diabetes of long duration and that there is a disturbance in the relative frequency of the different types of islet cells, with a relative decrease in the insulin producing β-cells. In the islet tissue of diabetic subjects β-cells show degenerative changes or signs of increased activity (Gepts, 1965).

The above observations naturally lead to the assumption that diabetes mellitus is a result of increased destruction of islet β-cells, or defective neogenesis of these cells, or a combination of the two.

The difficulty in obtaining human material for diabetes research has led to the development of different experimental systems, among which induction of diabetes in laboratory rodents with β-cytotoxic substances (cf. Rerup, 1970) and the use of animals with spontaneous hereditary diabetes (Renold and Burr, 1970) are of great importance.

Of the β-cytotoxic substances known at the present time streptozotocin (Fig. 1), which was first reported to be diabetogenic by Rakieten, Rakieten and Nadkarni (1963), has been the most widely used during the last decade for inducing experimental diabetes in laboratory animals (cf. Rerup, 1970; cf. Rudas, 1972). Streptozotocin consists of a 2-deoxy-D-glucose and an N-nitrosomethylurea

residue (Herr et al., 1967). It has been claimed that N-nitrosomethylurea is non-diabetogenic and that addition of glucose to this residue confers the ability to damage the pancreatic islet β-cells (Schein and Loftus, 1968). However, N-nitrosomethylurea alone has recently been shown to damage islet β-cells of mice (Gunnarsson, Berne, and Hellerström, 1974).

It has been pointed out that the nitrosamines (one of which is N--nitrosomethylurea), which are carcinogenic, mutagenic and sometimes teratogenic (Magee and Barnes, 1967; Druckrey et al., 1967), are present in low concentrations in various food products (Crosby et al., 1972) or can be synthesized endogenously from amine precursors and nitrite (Sander, 1971). Against this background it is possible that N-nitrosomethylurea also is noxious to human islet β-cells (Berne et al., 1974) and therefore the diabetogenic properties of this substance need to be further investigated.

Regarding spontaneous hereditary diabetes, the variety occurring in the Chinese hamster (Meier and Yerganian, 1959) is considered to be the most like human diabetes mellitus (Renold and Burr, 1970). The diabetic syndrome in Chinese hamsters is thus characterized by islet atrophy and a relative decrease in the proportion of insulin producing β-cells in the islets. It has been considered that the islet atrophy is a primary æ tiologic mechanism in the evolution of this diabetic syndrome (Carpenter et al., 1967). Although spontaneous diabetes in Chinese hamsters is considered to be hereditary, it is possible that environmental factors play a role in the development of this syndrome (Yerganian, 1964).

The chief aims of the present investigation were to study the following:

1. the β-cytotoxic and diabetogenic actions of streptozotocin in non-diabetic Chinese hamsters;
2. the β-cytotoxic and diabetogenic actions of the N-nitrosomethylurea moiety of the streptozotocin molecule in non-diabetic Chinese hamsters;
3. the mode of action of streptozotocin and N-nitrosomethylurea on the pancreatic islet cell β-cells;
4. the reserve volume and capacity for regeneration of the pancreatic islets in experimentally diabetic Chinese hamsters;
5. the morphological events of islet cell neogenesis in the pancreatic tissue of experimentally diabetic animals.

MATERIAL AND METHODS

1. Animals

Non-diabetic Chinese hamsters (I-VI), mice (VI) of the C57-Bl strain and mice with recessively inherited obese hyperglycaemia (one obese and one lean litter) of both sexes were used. The Chinese hamsters belonged to an inbred stock kept at the Institute of Pathology in Umeå since 1964. They emanated originally from Dr. Yerganian's colony in Boston, USA. They were selected at random from families in which no case of overt diabetes had occurred for more than five generations. All animals were fed a conventional diet for laboratory rodents (Anticimex, Sollentuna, Sweden) and were given tap water *ad libitum*.

2. Chemicals

Streptozotocin: (I-III) (Received as a gift from Dr. W.E. Dulin, The Upjohn Co., Kalamazoo, Michigan 49001, USA). As a rule strepto-

zotocin was dissolved in a citrate-phosphate buffer (McIlvaine, 1921), pH 4.0 (Garret, 1960), at a concentration of 2% (w/v) and was injected intraperitoneally.

N-nitrosomethylurea: (IV-VI) (Pfaltz and Bauer, Flushing, New York, USA). N-nitrosomethylurea was dissolved in a citrate-phosphate buffer (McIlvaine, 1921), pH 4.0 (Swann, 1968), at a concentration of 2% (w/v) and was injected intraperitoneally.

^{3}H-N-nitrosomethylurea: (VI) (labelled at the methyl group, spec. act. 48 mCi/mM, New England Nuclear, Boston, USA). [illegible]-methylurea was dissolved in a citrate-phosphate buffer (McIlvaine, 1921), pH 4.0 (Swann, 1968), at a concentration of 5% (w/v) and injected intravenously.

Nicotinamide: (II; IV and VI) (AB Kebo, Stockholm, Sweden). Nicotinamide was dissolved in distilled water at a concentration of 10% (w/v) and injected intraperitoneally.

^{3}H-Thymidine: (III) 5Ci/mM (The Radiochemical Centre, Amersham, England). The substance was injected intraperitoneally.

3. Blood glucose determinations

Blood samples (I-IV and VI) were taken from non-fasting animals by the orbital bleeding technique (Riley, 1960) under ether anæsthesia. Blood glucose determinations were performed by the glucose-oxidase method, using a commercially available kit (AB Kabi, Stockholm, Sweden). The error of blood glucose estimation was calculated from the difference between duplicates, using the formula

$$\frac{(x'_2 - x'_2)^2 + (x''_1 - x''_2)^2 + \ldots (x^{(n)}_1 - x^{(n)}_2)^2}{2n}$$

where x_1 and x_2 signified a duplicate sample and n was the total

number of duplicate determinations (Falkmer, 1961). The numerical value of the error was 3.4 mg/100 ml.

Clinistix^{R} was used to test for glucosuria and Ketostix^{R} for ketonuria.

4. Partial pancreatectomy

In ten non-diabetic female Chinese hamsters of the same litter, weighing 26-31 gm, an incision was made through the abdominal wall under ether anæsthesia and a partial pancreatectomy was performed. The amount of pancreas excised varied in different animals. The operation was performed from the splenic part towards the head of the pancreas. In the animal from which the largest piece of pancreas was removed about 30% of the pancreas was left in the abdomen. One animal was only sham-operated. Blood samples for glucose analyses were taken before and during the experimental period, which comprised nine days, after which time all animals were sacrificed. Both the excised pancreas and the rest of the pancreas, which was dissected free at sacrifice, were weighed on a Mettler K7 balance after fixation in Bouin's fluid. Three animals died during the experimental period.

5. Autoradiography

To obtain whole body autoradiograms (VI) the animals were anæsthetized with ether and killed by freezing in hexane cooled with solid CO_2 to $-78^{\circ}C$. After freezing the animals were embedded in a mixture of carboxy-methyl cellulose and water. Autoradiography was then performed as described by Ullberg (1954, 1958). Thus sagittal sections (20 µ thick) of the whole animals were cut on tape (Minnesota Mining and Manufacturing Co., USA, tape No. 800) at $-15^{\circ}C$ in a microtome. The sections were freeze-dried at $-15^{\circ}C$ and autoradiograms

were obtained by attachment of the sections to photographic plates (G5 Ilford). After exposure the photographic plates were developed in Kodak D19 and fixed in Gevaert G305 A. The sections were stained in hematoxylin-eosin for identification in the autoradiograms.

For microautoradiographic studies (VI) the ^{3}H-nitrosomethylurea treated animals were killed by cervical dislocation. Pieces of the pancreas were quickly removed, frozen in isopentane, cooled with liquid nitrogen, freeze-dried at -40°C in a Speedivac-Pearse tissue dryer for 5 days and then embedded in paraffin. 5-7 µ thick sections were then cut on tape (MMM No. 668) and microautoradiography was performed according to Hammarström et al. (1965). This included attachment of the tape sections to photographic plates (K2 and G5 Ilford), exposure at -10°C and removal of the tape from sections with xylol. The photographic plates were developed for 3 minutes in Kodak D19, fixed, and then stained with hematoxylin-eosin and in some cases also with aldehyde fuchsin according to Jennings (1965), as described by Tjälve (1971), for identification of the β-cells in the pancreatic islets.

For microautoradiographic study of the pancreas in ^{3}H-thymidine injected animals specimens were taken after sacrifice, fixed in Bouin's fluid, dehydrated, embedded in paraffin and sectioned into 4 µ thick sections. The conventional liquid emulsion method (Ilford K2 diluted 2:1 with redistilled water at 45°C) was used for the microautoradiography. After exposure and development (Kodak D19) the sections were stained with Ehrlich's hematoxylin.

6. Light microscopy

For fixations, 10% (v/v) formalin or Bouin's fluid (I-V) was most often used. The specimens were embedded in paraffin or in butoxy

ethanol-glycol-methacrylate (AB Lambda, Stockholm, Sweden) (IV). The paraffin embedded material was sectioned into about 4 µ thick sections and the methacrylate embedded material into 1 µ thick sections (Sorvall IB-4 microtome). The following stains were mainly used: hematoxylin-eosin, van Gieson's stain, PAS, aldehyde fuchsin - sometimes counterstained with Ponceau-fuchsin (Hultquist, 1962), chrome alum hematoxylin, toluidine blue (1% pH 7.0), alkaline Congo red (Puchtler et al., 1962) and silver impregnations according to Hellerström and Hellman (1960) and Grimelius (1968).

7. Volumetric quantitation of islet tissue

For quantitation (II) of the total pancreatic islet volume the whole pancreas was carefully excised in sacrificed Chinese hamsters. It was fixed in Bouin's fluid, weighed (Mettler K7), dehydrated, paraffin-embedded and serially sectioned into alternately 4 x 7 µ and 7 x 4 µ sections. The 7 µ thick sections were stained with van Gieson's stain and the pancreatic islet areas in the sections were measured planimetrically at a magnification of x 400. The distance between the sections was 224 µ. The total islet volume in each animal and the mean islet diameter were calculated by conventional methods (Hellman, 1959).

8. Electron microscopy

Specimens taken from the pancreas for electron microscopic examination (I and V) were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer or in 2.34 M veronal acetate buffer adjusted to pH 7.4 and post-fixed in 1% osmium tetroxide. The specimens were dehydrated and embedded in Epon 812 (Luft, 1961). Toluidine-blue-stained approximately 1 µ thick sections were examined in a light microscope

for the identification of islets. Ultrathin sections were prepared with an LKB Ultrotome, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and viewed in a Siemens 1A and 101 and a Zeiss EM 9 electron microscope.

RESULTS AND DISCUSSION

1. Toxicity of streptozotocin and N-nitrosomethylurea

Since the L.D. 50 for N-nitrosomethylurea (mol. weight 103) at an observation time of 48 hours was 125 mg/kg body weight, it seems to be considerably more toxic in Chinese hamsters than streptozotocin (mol. weight 265), which has an L.D. 50 value of 400 mg/kg body weight (I and IV). It is reasonable to assume that the addition of glucose to the N-nitrosomethylurea moiety lowers the toxicity of this substance. In whole body autoradiography of mice labelled N-nitrosomethylurea was found to be distributed in the brain (Kleimhues and Patzschke, 1971), and in rats N-nitrosomethylurea has been shown to penetrate the central nervous system and produce neurological disturbances (Schein, 1969). It may thus be suggested that N-nitrosomethylurea penetrates the blood-brain barrier but that streptozotocin does not. This would at least partly explain the differences in toxicity between the two substances. A study of the distribution of labelled streptozotocin in a whole body autoradiogram would probably give further information regarding this problem.

2. Blood glucose variations evoked by streptozotocin and N-nitrosomethylurea

The blood glucose variations evoked by intraperitoneal injection

of streptozotocin 200 mg/kg body weight (I) or N-nitrosomethylurea 50 mg/kg body weight (IV) in Chinese hamsters were very similar. Thus an initial hyperglycaemic peak was recorded in both instances and a decrease at 6 hours, followed by moderately or markedly elevated values during the following days. The initial blood glucose elevation can be explained by inhibition of insulin release, an effect which both streptozotocin (Golden et al., 1971) and N-nitrosomethylurea (Gunnarsson et al., 1974) exhibit in isolated islets. The hypoglycaemia after 6 hours is obviously caused by insulin liberation from β-cells, which at that time are undergoing disintegration. The marked transient hyperglycaemia observed in streptozotocin-diabetic Chinese hamsters after one day (II) was not found in the N-nitrosomethylurea injected animals (IV). No simple explanation for this difference can be offered at present. The results obtained show that the glucose residue of the streptozotocin molecule is not necessary for induction of hyperglycaemia in Chinese hamsters.

The finding that N-nitrosomethylurea alone is able to cause hyperglycaemia and glucosuria and to damage the pancreatic islet β-cells (see below) is interesting, since this substance occurs in the environment (Crosby et al., 1972) and environmental factors are probably of importance in the pathogenesis of human juvenile diabetes mellitus, according to Tattersall and Pyke (1972).

The high mortality (90% after 9 days) in the diabetic animals after injection of N-nitrosomethylurea (IV) prevented longer periods of observation of the blood glucose level. However, no obvious decrease in this level occurred during the experimental period. Of the streptozotocin-diabetic Chinese hamsters about 50% lived to the end of an experimental period of 3 months (II). No signs of reduction of the blood glucose concentration were noted in the diabetic animals,

indicating that as in human diabetes mellitus there was no regression of the diabetic state.

3. Islet cell destruction by streptozotocin and N-nitrosomethylurea

Both streptozotocin (I) and N-nitrosomethylurea (IV) had a toxic effect on the islet β-cells of Chinese hamsters. Obviously the destruction of the insulin producing β-cells is the mechanism underlying the diabetogenic action of streptozotocin and N-nitrosomethylurea. Beside the β-cytotoxic effect both substances were noxious to the argyrophil α_2- and α_1-cells of the islet tissue. On the ultrastructural level the changes in β-cells and α-cells occurred at the same time after injection of both streptozotocin and N-nitrosomethylurea, indicating that the effect on the α-cells was probably not secondary to the destruction of β-cells.

In other animal species streptozotocin is reported to be specifically toxic to β-cells (cf. Rerup, 1970), with one exception, namely the rabbit, in which α_2-cells but not α_1-cells were damaged (Lazarus and Shapiro, 1972).

In mice β-cell destruction could only be induced with very high doses of N-nitrosomethylurea (230 mg/kg body weight), giving a very high mortality which rendered it impossible to determine whether hyperglycaemia occurred in these animals (Gunnarsson et al., 1974). Thus there seem to be species differences in the cytotoxic effects of streptozotocin and N-nitrosomethylurea. From the present results it would seem that non-diabetic Chinese hamsters apparently are very suitable animals for studies of the β-cytotoxic and diabetogenic environmental nitrosamines.

The β-cytotoxic effect of streptozotocin has been utilized in the treatment of insulin producing malignant insulomas (Murray-Lyon et al., 1968). Feldman et al. (1972) reported recently that strepto-

zotocin was effective in the treatment of two cases of malignant carcinoids, one in the lung and one with an unknown primary localization. This information is interesting since the islet α_2-cells, which are destroyed by streptozotocin in the Chinese hamster, are argyrophil with the silver impregnation method of Grimelius (1968), and this is also true of most carcinoids in the gastrointestinal tract and some bronchial carcinoids in humans (Wilander and Grimelius, unpublished observations). This may indicate that the argyrophil cells of Chinese hamsters are more like human argyrophil cells than are the argyrophil cells of other laboratory rodents, with the exception of the rabbit (Lazarus and Shapiro, 1972).

4. Action of streptozotocin and N-nitrosomethylurea on islet tissue

Similarities exist between streptozotocin and N-nitrosomethylurea in that both substances cause a depletion of nicotinamide adenine dinucleotide (NAD) in pancreatic islet cells (Ho and Hashim, 1972; Hinz et al., 1973; Schein et al., 1973; Gunnarsson et al., 1974). Both the diabetogenic and the NAD lowering effect of streptozotocin can be inhibited by pre-treatment with nicotinamide, but not with nicotinic acid (Schein et al., 1967; Dulin and Wyse, 1969; Stauffacher et al., 1970; Ho and Hashim, 1972; Hinz et al., 1973; Schein et al., 1973). In the Chinese hamster the induction of diabetes by streptozotocin and also by N-nitrosomethylurea can be inhibited by nicotinamide, 500 mg/kg body weight (II and IV), indicating that depletion of cellular NAD may be causally related to the β-cell deterioration by these substances in this animal. Nicotinic acid and nicotinamide are both precursors in the synthesis of NAD (Ichiyama et al., 1967), which plays a central role in the energy metabolism of all cells. Two pathways for the biosynthesis of NAD are currently believed to be predominant in mammals. Preiss and Handler (1958) have described

a pathway by which nicotinic acid is converted to NAD via nicotinic acid mononucleotide and nicotinic acid adenine dinucleotide. More recently Dietrich et al. (1966) have shown that mammalian tissues can convert nicotinamide to NAD. At the present time the physiological role of these two pathways of NAD synthesis is uncertain. However, a recent report by Collins and Chaykin (1972) indicated that nicotinamide was a clearly better precursor of NAD than nicotinic acid in most tissues and that nicotinic acid could be converted rapidly to nicotinamide for NAD synthesis. It seems reasonable to presume that in islet tissue, also, nicotinamide is a more important precursor of NAD synthesis than nicotinic acid, as the former, but not the latter, can prevent the induction of diabetes by streptozotocin (Schein et al., 1967; Dulin and Wyse, 1969; Stauffacher et al., 1970; Ho and Hashim, 1972; Hinz et al., 1973; Schein et al., 1973). A study of the uptake of nicotinic acid and nicotinamide in islet tissue of mice and Chinese hamsters is in progress and should give further information regarding this problem.

It has been shown that labelled N-nitrosomethylurea accumulates in the pancreatic islets of Chinese hamsters but not in those of mice (VI). Thus it seems as if the ability of N-nitrosomethylurea to be specifically taken up by islet tissue reflects its ability to produce islet β-cell damage and diabetes, since in Chinese hamsters a dose of 50 mg/kg body weight destroys all types of islet cells and causes hyperglycaemia, while a dose of 150 mg/kg body weight of N-nitrosomethylurea causes no blood glucose elevation in mice (VI). The reason for this difference in accumulation and ability to produce diabetes is obscure, but it may reflect some difference in the intracellular metabolism or transport system of the islet tissue between mice and Chinese hamsters.

Pre-treatment with nicotinamide did not prevent the accumulation of labelled N-nitrosomethylurea in the pancreatic islets of Chinese hamsters (VI). Thus nicotinamide obviously does not exert its protective action against N-nitrosomethylurea by altering the ability of the latter to reach its target in the cells.

In the pancreatic islets of Chinese hamsters the uptake of labelled N-nitrosomethylurea in β- and α-cells was similar (VI). This finding was in accordance with the observation at light and electron microscopy after injection of N-nitrosomethylurea, that the degenerative changes seemed to affect both types of cells equally and at the same time. However, whether the noxious action of N-nitrosomethylurea on pancreatic islet α-cells is mediated via a decrease in cellular NAD, as is probable in the β-cells, is not known at present time.

Within the islets the highest label was found over the nuclei of the cells (VI), indicating interference by N-nitrosomethylurea with some nuclear component. N-nitrosomethylurea is an alkylating carcinogen, causing preferentially 7-alkylation of guanine in DNA and RNA (cf. Pegg, 1973). On the other hand, the action of many alkylating agents takes place at some stage of the NAD biosynthesis (Kun et al., 1964). NAD biosynthesis is confined to the nuclei of the cells (Siebert et al., 1965). The protective action of nicotinamide against the islet cell cytotoxicity caused by N-nitrosomethylurea (IV) and streptozotocin (II) suggests that destruction of islet cells by these substances takes place through a decrease in the nicotinamide available for synthesis of NAD. An effect on both nuclear DNA-RNA and NAD biosynthesis seems possible as an explanation for the cell damage, but the mechanism involved must be investigated further.

5. Islet cell regeneration in streptozotocin-diabetic animals

Opinions are still controversial as to the mode of pancreatic islet neoformation. It seems reasonable to suggest that islet cell neogenesis to some extent mirrors the foetal pancreatic islet development. However, it is not clear whether the endocrine cells arise from separate stem cells, possibly neuroepithelial cells implanted in the gastrointestinal tract in early embryonal life, as postulated by Pearse et al., (1973), or from common progenitor cells with an ability to differentiate in an exocrine or endocrine direction. A transformation of exocrine cells to endocrine cells has also been speculated upon as a possible mode of islet formation; this was first suggested by Laguesse (1895).

The differentiation of endocrine cells in the pancreas is in all probability regulated by hormones and metabolites. Support for this opinion is given by the finding of an increase in the relative proportion of endocrine tissue in foetuses as a result of maternal diabetes (cf. Hultquist, 1970) and of a decrease (in mice) on injection of insulin (Frye, 1958). Whether or not high blood glucose *per se* is able to stimulate β-cell proliferation is unclear. Contradicting this theory is the observation of van Assche (1969) that in infants with diabetic mothers and with acrania and a defective hypophyso-hypothalamic system no islet hyperplasia is observed. However, an increase in β-cell replication *in vitro* at a high glucose concentration has been reported in pancreases from newborn rats (Chick et al., 1973) and adult mice (Andersson, 1973). The permanent hyperglycaemia recorded in the streptozotocin-diabetic Chinese hamsters directly or indirectly stimulated islet cell neoformation, since a higher incorporation of labelled thymidine was seen in the diabetic animals than in normal controls (III). At the same time "clear cells"

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were observed in the islet tissue, suggesting that these cells are involved in islet cell regeneration. Such cells were also found in the exocrine parenchyma, sometimes in association with tubular structures. The close relationship between islet cell regeneration and "clear cells" has been pointed out earlier (Boquist, 1968a and 1968b; Edström, 1973).

Injection of alloxan in non-diabetic Chinese hamsters causes a transitory diabetic state with return to normal conditions within a few weeks. The normalization seemed to be a result of neoformation and restoration of the pancreatic islet tissue (Boquist, 1968a). The permanent hyperglycaemia as well as the marked islet atrophy in the streptozotocin-diabetic animals (II) after one month indicate that streptozotocin diabetes in Chinese hamsters is irreversible, in contrast to alloxan diabetes in this animal. The reason for this difference is not known. Streptozotocin has been shown to inhibit the progression of cells to mitosis (Bhuyan, 1970). This may have some significance for the obtained results. On the other hand, streptozotocin, but not alloxan, damages the islet α_2-cells. Since α_2-cells are the first to be detected in the pancreas during early embryonal life, it has been suggested that β-cells are developed by transformation of α_2-cells (cf. Pictet and Rutter, 1972). This theory could partly explain a difference in regeneration of islet tissue in streptozotocin- and alloxan-diabetic Chinese hamsters, since the noxious action of streptozotocin on α_2-cells would destroy the matrix for β-cell neoformation.

It has been claimed that the pancreatic β-cells have a limited potential for sustained regeneration (Brosky and Logothetopoulos, 1969; Like and Chick, 1970). This observation also seemed to be valid in the present study, as the morphological signs of regeneration of islet tissue declined towards the end of the experimental

period (III). This question is of great importance regarding human juvenile diabetes. However, whether the islet atrophy in these cases is a result of a defective capacity for β-cell regeneration or of exhaustion of the normal but limited capacity for β-cell neoformation is not known.

In streptozotocin-diabetic Chinese hamsters large islets were seen at light microscopy. These islets consisted of endocrine cells with a large nucleus and a prominent nucleolus; varying degrees of vacuolization in the cytoplasm and also swelling were seen. Necrosis of what at least may have been partly α_2-cells was also found in the periphery of these islets (III). An increase in nuclear size suggests increased protein synthetic activity (Oehlert and Schultze, 1960; Hultquist, 1962; Patent and Alfert, 1967), in the Chinese hamsters studied here sometimes probably resulting in exhaustion. Hypertrophy of the islets was evident a few weeks after injection of streptozotocin and was seen in all diabetic animals after one month (III). Gepts (1965) has described signs of increased activity of β-cells in human juvenile diabetes, and Unger et al. (1970) have reported strikingly elevated plasma glucagon levels in diabetic patients with severe keto-acidoses. It seems as if an effect of increased activity of islet β-cells is caused by an increased demand for insulin in the remaining β-cells as a result of insufficient β-cell regeneration. However, the mechanism underlying the increase in α_2-cell activity remains unclear.

6. Effect of pancreatectomy

The weight of the partially excised pancreas and the weight of the rest of the pancreas taken out at sacrifice in 7 Chinese hamsters are given in Table I. As seen in Figure 2, all partially pancreatectomized animals were hyperglycaemic even when a relatively small portion

of the pancreas had been resected. Only the sham-operated hamster was normoglycaemic. A strong correlation was observed between the mean blood glucose elevation and the weight of the part of the pancreas remaining in the abdomen after partial pancreatectomy (r -0.97 $p < 0.001$) (Fig. 3). No correlation was found between the weight of the excised pancreas and the mean blood glucose elevation (r 0.57 $p > 0.1$) The results indicate that the reserve volume of islet tissue varies in different animals but on the whole is very small. Knowledge of the reserve volume of islet tissue probably is important as this volume must influence the noxious action of β-cytotoxic substances on the development of diabetes. Since Tattersall and Pyke (1972) have observed that the hereditary trait for human diabetes mellitus is not very strong, the pathogenesis of this disease should possibly be sought among environmental factors. However, it must be considered that there may be a hereditary trait influencing the total amount of islet tissue in the pancreas of humans as well as of experimental animals and that this probably has influence on the ability to develop diabetes. The importance of this argumentation is evident from the observation that both the normal and atrophic islet volume in normal and in spontaneously diabetic Chinese hamsters is hereditary (Carpenter et al., 1967 and 1970).

GENERAL SUMMARY

The results of the present investigations can be summarized as follows.

1. The cytotoxic action of streptozotocin on the pancreatic islet β-cells of Chinese hamsters plays a major role in the development of streptozotocin-diabetes in this animal.

2. The glucose moiety of the streptozotocin molecule is not necessary for the induction of diabetes in the Chinese hamster, since N--nitrosomethylurea alone has been found to be β-cytotoxic and diabetogenic. N-nitrosomethylurea belongs to the nitrosamines, which occur in the environment, and attention must now also be directed towards a possible diabetogenic effect of these substances in humans.

3. The general toxicity of N-nitrosomethylurea decreases when glucose is added to the molecule.

4. The diabetogenic effects of streptozotocin and N-nitrosomethylurea can be prevented by pre-treatment with nicotinamide, indicating that the modes of action of the two diabetogenic substances are similar.

5. The diabetogenic substance N-nitrosomethylurea was selectively accumulated in the pancreatic islets of Chinese hamsters but not of mice. The ability of N-nitrosomethylurea to be specifically taken up by islet tissue seems to reflect its ability to produce islet cell damage, since a dose of 50 mg/kg caused diabetes in Chinese hamsters while a dose of 150 mg/kg was not diabetogenic in mice.

6. Pre-treatment with nicotinamide did not prevent the specific uptake of N-nitrosomethylurea in the pancreatic islets of Chinese hamsters. Consequently the protective function of nicotinamide is obviously not due to changes in the ability of N-nitrosomethylurea to reach its target in the islet tissue.

7. Streptozotocin and its aglucone N-nitrosomethylrea are also cytotoxic to the pancreatic islet argyrophil α_2- and α_1-cells.

8. The irreversible nature of the streptozotocin-induced diabetic syndrome in Chinese hamsters is a result of a limited capacity for islet cell neoformation, resulting in permanent islet atrophy, as in the spontaneous hereditary diabetic syndrome in Chinese hamsters and human juvenile diabetes mellitus.

9. The occurrence of "clear cells" in islet tissue and in exocrine pancreas, especially in association with tubular structures, with all probability constitutes a sign of islet cell neoformation.

10. The reserve volume of the pancreatic islets in non-diabetic Chinese hamsters varies but on the whole is small, indicating that not all animals are equally sensitive to β-cell depletion.

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TABLE I. Mean blood glucose concentration (mg/100 ml) and pancreatic weight (gm) in partially pancreatectomized Chinese hamsters.

Animal No.	Mean blood glucose concentration M ± SEM	Weight of excised pancreas	Weight of remaining pancreas	Total pancreatic weight
228	178 ± 5	0.0270	0.0745	0.1015
229	171 ± 9	0.0910	0.0565	0.1475
230	161 ± 4	0.1536	0.0803	0.2339
231	176 ± 7	0.1406	0.0592	0.1998
232	145 ± 3	0.0797	0.1257	0.2054
233	137 ± 4	0.0611	0.1587	0.2198
234[x]	102 ± 3	0.0000	0.2066	0.2066

[x] sham-operated

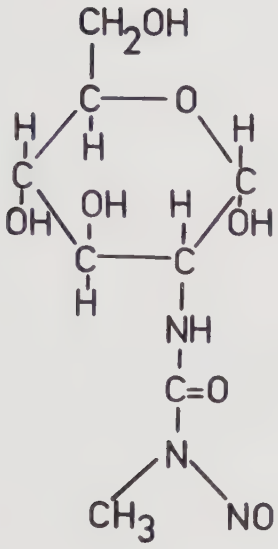

Fig. 1.

Fig. 1. Structural formula of streptozotocin.

Fig. 2. Blood glucose concentrations (mg/100 ml) in 6 partially pancreatectomized Chinese hamsters and one sham-operated control. The horizontal dash-dot lines represent the limits of mean blood glucose concentration $\pm$ SE x t 0.05 in normal hamsters.

Fig. 3. Correlation between mean blood glucose concentration (mg/100 ml) and weight of remaining pancreas (gm) in partially pancreatectomized Chinese hamsters and one sham-operated control.

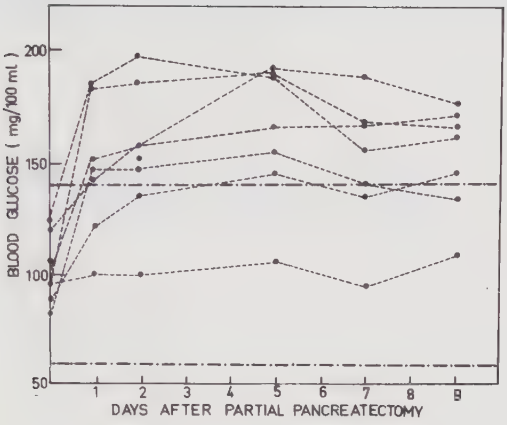
BLOOD GLUCOSE (mg/100 ml)
200
150
100
50
1
2
3
4
5
6
7
8
9
DAYS AFTER PARTIAL PANCREATECTOMY

Fig. 2.

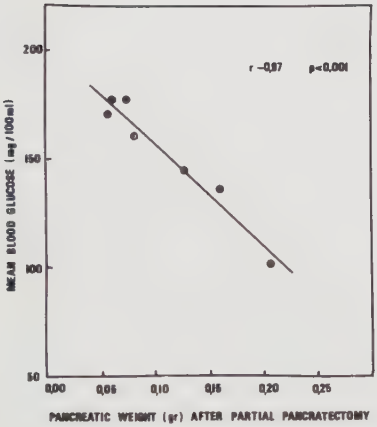
r -0,87
p<0,001
MEAN BLOOD GLUCOSE (mg/100ml)
200
150
100
50
0,00
0,05
0,10
0,15
0,20
0,25
PANCREATIC WEIGHT (gr) AFTER PARTIAL PANCRATECTOMY

Fig. 3.

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Streptozotocin-Diabetes in the Chinese Hamster*

Blood Glucose and Structural Changes During the First 24 Hours

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Summary

Intraperitoneal administration of 200 mg/kg body weight of streptozotocin to non-diabetic adult Chinese hamsters, evoked a tri-phasic blood glucose response. There was a hyperglycemic peak after 3 hours and a hypoglycemic phase with a minimum at 6 hours, after which time there was a rather rapid return to hyperglycemia. Light and electron microscopic examinations during the first 24 hours after the injection of streptozotocin showed pyknosis, vacuolization, marked degeneration and necrosis of the β-cells. Degenerative and necrotic changes were also seen in a few α_2-cells. The results denote that in the Chinese hamster streptozotocin has an early and direct cytotoxic action on the β-cells and probably also on a few α_2-cells.

Key-Words: *Chinese Hamster Streptozotocin Pancreatic Islets β-Cell Degeneration α_2-Cell Degeneration Hyperglycemia*

Introduction

Streptozotocin possesses antibiotic, diabetogenic and both anti-tumoral (*Evans, Gerritsen, Mann and Owen* 1965) and tumor-inducing (*Arison and Feudale* 1967) properties. It was first found to induce diabetes in laboratory animals by *Rakieten, Rakieten and Nadkarni* (1963) and numerous subsequent reports have confirmed its diabetogenic effects in mammalian (*Alexander, Britton, Cohen, Mashiter, Nixon and Smith* 1971, *Arison, Ciaccio, Glitzer, Cassaro and Pruss* 1967, *Brosky and Logothetopoulos* 1969, *Golob, Rishi, Becker and Moore* 1970, *Junod, Lambert, Orci, Pictet, Gonet and Renold* 1967, *Junod, Lambert, Stauffacher and Renold* 1969, *Losert, Rilke, Loge and Richter* 1971, *Orci, Junod, Renold and Rouiller* 1968, *Pitkin and Reynolds* 1970, *Rerup and Tarding* 1969, *Richter, Loge and Losert* 1971, *Schein and Bates* 1968, *Schein, Cooney and Vernon* 1967, *Sybulski and Maughan* 1971) and non-mammalian (*Thomas* 1971) species. Streptozotocin has been used for the preparation of β-cell deficient islets for microchemical and metabolic studies in guinea pigs (*Howell, Edwards and Whitfield* 1971, *Petersson, Hellerstrom and Gunnarsson* 1970) and for the treatment of insulin producing islet cell tumors in man (*Arnould, Ooms and Bastenie* 1969, *Blackard, Garcia and Brown* 1970, *Murray-Lyon, Eddleston, Williams, Brown, Hogbin, Bennett, Edwards and Taylor* 1968, *Sadoff* 1969, *Smith, Stoll, Vance, Ricketts and Williams* 1971, *Taylor, Schwartz, Zannini and Ryan* 1970).

There are species differences in the response to streptozotocin (*Rerup* 1970) and some of its effects are similar to those of alloxan (*Veleminsky, Burr and Stauffacher* 1970). Because of this, it would be preferable to perform streptozotocin studies in a species in which the effects of alloxan are known, and still better in a species in which also spontaneous diabetes mellitus of pancreatic origin occurs. One such species is the Chinese hamster (*Boquist* 1969b) in which the disease is considered to be very similar to human diabetes.

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The present report, representing a part of our studies on the relationship between exogenous and endogenous diabetes, is confined to the blood glucose variations and morphologic alterations evoked within the first 24 hours after streptozotocin administration.

Material and Methods

Non-diabetic Chinese hamsters of both sexes were used. They were 3-14 months old and weighed 17-39 gm and were selected at random from families in which no cases of overt diabetes had occurred since at least six generations. Before the experiments the hamsters were controlled to be free from glucosuria and hyperglycemia. They were fed a conventional diet for laboratory rodents and were given tap water *ad libitum*.

In a first experiment, streptozotocin (gift from the Upjohn Comp., Kalamazoo, Michigan) was administered intraperitoneally at a dose of 200, 400, 600 and 800 mg/kg body weight to 20 non-fasting Chinese hamsters. Immediately before the use, it was dissolved in a citrate-phosphate buffer at pH 4.0 and a concentration of 4%. Then a rough estimation of L.D. 50 was performed at 48 hours' observation time. In the ensuing main experiments streptozotocin was injected in 102 non-fasting Chinese hamsters at a dose of 200 mg/kg body weight and a concentration of 2% (the volume of a 2% solution was easier to inject than that of a 4% solution). Sixteen control hamsters were injected with a corresponding volume of plain buffer. Blood from the orbital venous plexus was obtained under ether anesthesia 3, 6 and 24 hours after the injection of streptozotocin. Blood glucose determinations were performed with a glucose-oxidase method and the presence of glucosuria was investigated by means of glucose-oxidase paper strips (*Boquist* 1969b).

Twenty-five animals were killed 1/2, 1 1/2, 3, 6 and 24 hours after the injection of streptozotocin and specimens were taken from the pancreas for light and electron microscopic examination. The specimens were fixed in Bouin's fluid or 10% formalin. The following light microscopic stains were used: van Gieson's stain, aldehyde-fuchsin, chrome-alum-hematoxylin, and silver impregnations according to *Hellerström and Hellman* (1960) and *Grimelius* (1968). At 24 hours, specimens were also taken from the liver and kidneys; those from the liver were cut on a cryostate-microtome and stained with Scharlach rot or fixed in 10% formalin and stained with van Gieson's stain; those from the kidneys were fixed in 10% formalin and stained with van Gieson's stain.

For electron microscopic examination pancreatic specimens were fixed by immersion in 2.5% glutaraldehyde in 2.34 M veronal acetate buffer adjusted to pH 7.4 and postfixed in 1% osmium tetroxide in the same buffer. Then the specimens were rinsed, dehydrated in ethanol and embedded in Epon 812. Toluidine-blue-stained thick sections were examined in a light microscope for the identification of the islets. The thin sections were stained with uranyl acetate and lead citrate and the sections were viewed in a Siemens Elmiscope 1A and 101.

Results

L.D. 50

The approximate L.D. 50 of streptozotocin at a concentration of 4%, was 400 mg/kg body weight at 48 hours observation time (Fig. 1). When 200 mg/kg body weight of streptozotocin was injected at a concentration of 2% there was no mortality after 48 hours, and blood glucose variations and structural changes in the islets were found in almost all animals.

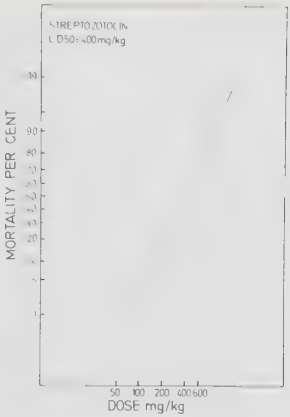

Fig. 1. Dose-mortality curve after intraperitoneal administration of streptozotocin, plotted on paper with log-probability scale.

Blood Glucose Variations

The mean level of blood glucose before the injections of streptozotocin was 101.9 ± 3.8 (mean value ± SE x $t_{0.05}$) in the experimental group and 103.0 ± 12.6 (mean value ± SE x $t_{0.05}$) in the control group. There was no significant difference in the mean blood glucose level between the two groups ($p > 0.05$). Nor could any sex (♂ 104.3 ± 6.1 and ♀ 100.5 ± 5.8; mean value ± SE x $t_{0.05}$; $p > 0.05$) or age (animals 3-8 months of age 99.5 ± 5.6 and animals 9-14 months of age 102.1 ± 5.7; mean value ± SE x $t_{0.05}$; $p > 0.05$) differences be recorded.

After the injection of streptozotocin a tri-phasic blood-glucose curve (Fig. 2) was obtained during the first 24 hours. There was a first hyperglycemic peak after 3 hours (272.1 ± 26.5; mean value ± SE x $t_{0.05}$) and a return to normoglycemia at 6 hours (103.3 ± 21.6; mean value ± SE x $t_{0.05}$). A second hyperglycemic phase was observed during the following hours with a maximum at 24 hours (277.2 ± 21.2; mean value ± SE x $t_{0.05}$). There were no significant blood-glucose variations in the control group.

All hyperglycemic animals had glucosuria 24 hours after the injection of streptozotocin.

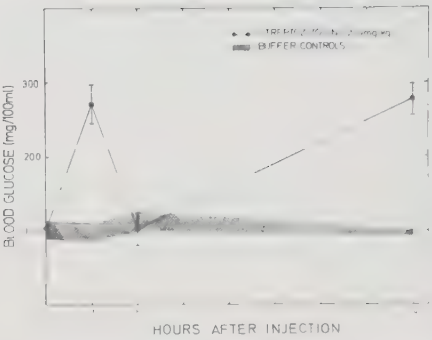

Fig. 2. Blood glucose variations in Chinese hamsters during the first day after the administration of streptozotocin. The heavy black line denotes the mean blood glucose level. The vertical bars indicate SE x $t_{0.05}$ of the blood glucose values at each observation time. The dashed area gives the range of SE x $t_{0.05}$ of the blood glucose level of control animals injected with plain buffer solution.

Light Microscopic Findings

At 1/2 hour and 1 1/2 hour after the injection of streptozotocin no obvious structural changes were seen in the islets.

Three hours after the injection pyknosis was seen in most β-cells (Fig. 3). Occasional β-cells exhibited cytoplasmic vacuolisation but there was no obvious degranulation. The α-cells were unaffected.

At 6 hours there were pyknosis, beginning nuclear fragmentation and marked cytoplasmic vacuolisation in the β-cells (Fig. 4). During the following hours many β-cells became necrotic with the appearance of amorphous aldehyde-fuchsin-positive debris. The structural changes varied in different islets and there were also variations from one animal to the other. A few α_2-cells were pyknotic at 6 hours and during the following hours necrosis occurred in a small number of the α_2-cells that were situated close to necrotic β-cells.

At 24 hours the islets possessed centrally situated amorphous slightly aldehyde-fuchsin-positive material

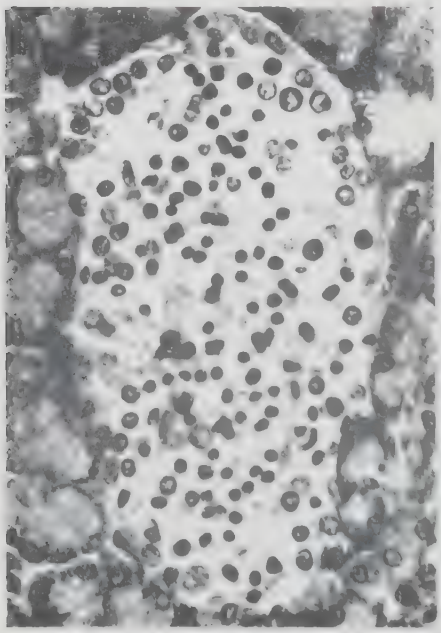

Fig. 3. Pancreatic islet of Chinese hamster 3 hours after the injection of streptozotocin showing pyknosis among the central β-cells. The peripheral α-cells are unaffected (van Gieson; x 520)

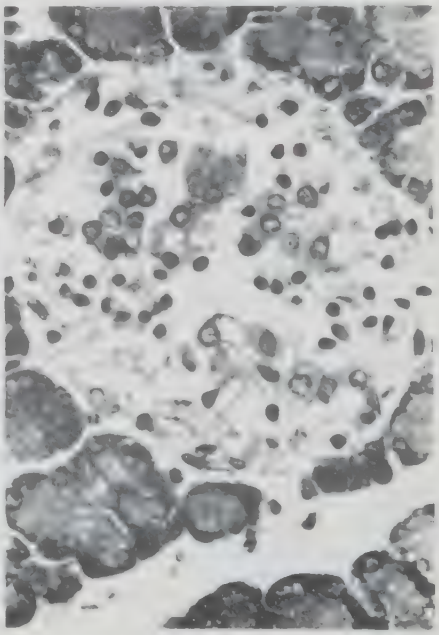

Fig. 4. Pancreatic islet of Chinese hamster 6 hours after the injection of streptozotocin showing pyknotic β-cells and marked intra- and extracellular vacuolization (van Gieson; X 520)

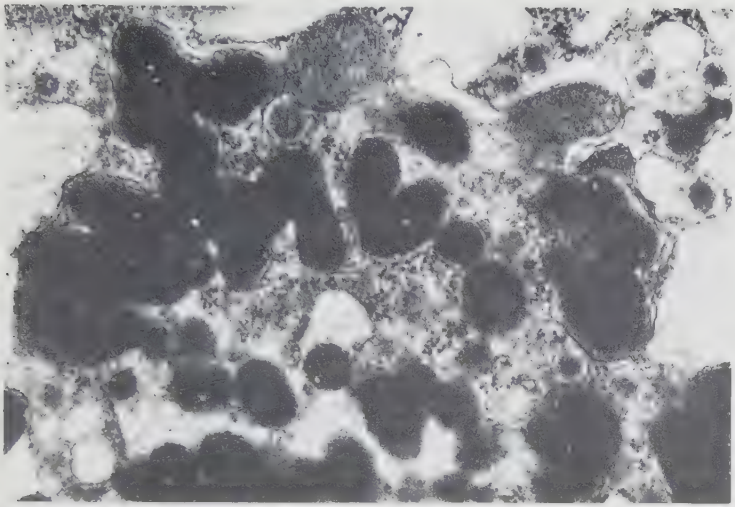

Fig. 5. Electron micrograph of β-cell of Chinese hamster 30 minutes after the administration of streptozotocin showing some typical secretory granules and aggreates of numerous granule cores, with or without associated membranes. (X 40,000)

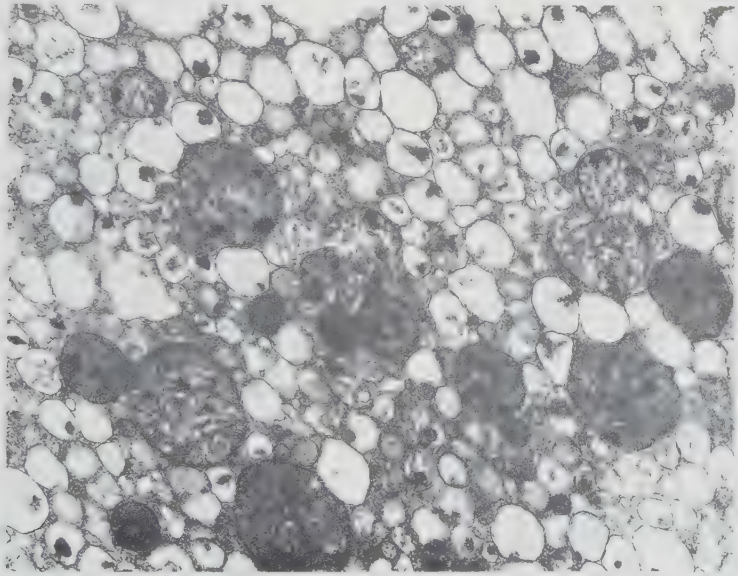

Fig. 6. Portion of β-cell 3 hours after the administration of streptozotocin showing secretory granules with preserved membranes and small electron dense irregular and thread-like particles, probably representing remnants of granule cores. Other granule sacs are completely electron lucent. The mitochondria are swollen and contain small electron dense particles. (X 19,000)

and a few peripheral endothelial cells and aldehyde-fuchsin-negative, non-argyrophil cells.

Localized infiltrates of lymphocytes were seen in the exocrine pancreas, often around the islets, at six hours and later. The number and size of these infiltrates varied considerably.

Slight fatty metamorphosis of the liver parenchymal cells was seen in about 50% of the animals at 24 hours. The kidneys were unaffected.

Electron Microscopic Findings

Thirty minutes after the injection of streptozotocin aggregates of secretory granules were seen in some β-cells (Fig. 5). The membranes around the secretory granules were occasionally disrupted. At 1 1/2 hour the β-cells exhibited cytoplasmic vacuoles of varying size, slight degranulation and swollen mitochondria.

At 3 hours pyknosis was seen in the β-cells. The secretory granules of some β-cells possessed preserved membranes but only small remnants of the granule cores which often were irregular and thread-like (Fig. 6). Other cores seemed to have disappeared, leaving only a small, membrane-bound, electron lucent vacuole.

From 6 hours until the end of the observation time marked degenerative alterations appeared in the β-cells, leading to necrosis and small remnants of necrotic debris. In a few less severely damaged β-cells there was lysosomal activity with signs of crinophagy (Fig. 7).

The α_1-cells were unaffected. Occasional α_2-cells exhibited lysosomes with incorporated secretory granules (Fig. 8). A few peripheral α_2-cells showed more marked degenerative changes (Fig. 9). Vacuoles and myelin figures were seen in the cytoplasm of these cells. Their secretory granules showed often preserved cores, and either disrupted or preserved membranes. In the latter case the granule often seemed to be swollen, leaving an electron dense small core surrounded by a large electron lucent space.

Discussion

The blood glucose variations evoked by streptozotocin administration to the Chinese hamster conformed to those observed in this species after alloxan injection (*Boquist* 1968a) in that a triphasic blood glucose curve was obtained after treatment with both these compounds. Differences exist also, mainly in that the first hypoglycemic peak came later after streptozotocin than after alloxan and that the hypo-

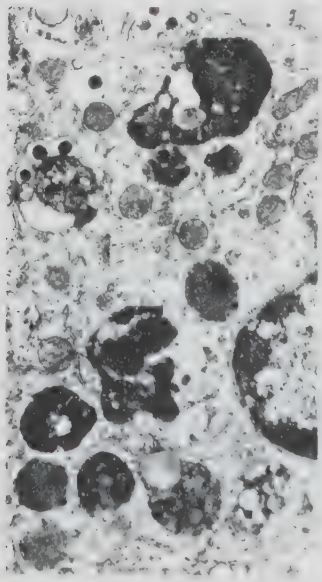

Fig. 7. β-cell 6 hours after streptozotocin injection containing numerous lysosomes with incorporated secretory granules. (X 11,000)

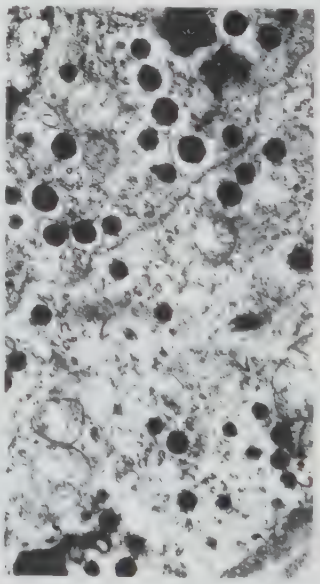

Fig. 8. Portion of two α_2-cells 6 hours after injection of streptozotocin showing typical secretory granules and lysosomes with incorporated granules. (X 8,000)

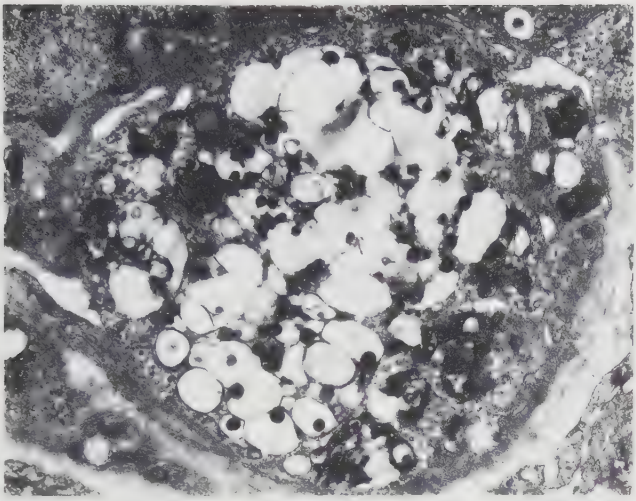

Fig. 9. Peripheral α_2-cell 24 hours after streptozotocin administration showing vacuoles, lysosomes with incorporated granules, condensed membranes, and secretory granules with disrupted or dilated membranes. (X 8,500)

glycemic phase was more pronounced in animals treated with streptozotocin than in those given alloxan. Moreover, the second hyperglycemic phase was characterized by higher blood glucose levels in the streptozotocin-treated animals than in those given alloxan. Differences in the blood glucose effects of streptozotocin and alloxan have also been recorded by others. Thus, conforming to our observations, *Rerup and Tarding* (1969) found a marked difference in the appearance of the first hyperglycemic peak after the two compounds, a more severe hypoglycemia after streptozotocin than after alloxan and an apparent irreversibility of streptozotocin.diabetes. These authors also observed that the plasma insulin and blood glucose levels were poorly correlated following streptozotocin treatment, in contrast to those in alloxan-treated animals. Sulphonylureas that do not affect alloxan diabetes, have an effect when given after streptozotocin (*Lundquist and Rerup* 1967, *Rerup and Tarding* 1969). Because of these differences it has been suggested that streptozotocin and alloxan produce different types of diabetes (*Rerup and Tarding* 1969). Ketosis and elevation of free-fatty-acid levels in plasma occur both after alloxan and streptozotocin (*Junod* et al. 1967) but the ketosis is less severe in streptozotocin-treated animals than those given alloxan (*Förster and Rudas* 1969, *Opie and Mansford* 1969). Streptozotocin, but not alloxan, has been found to deplete the nicotinamide adenine dinucleotide (NAD) levels in mouse liver (*Schein and Loftus* 1968).

Losert et al. (1971) have reported species differences in the sensitivity to streptozotocin. These authors induced severe diabetes mellitus in Chinese hamsters by intravenous injection of streptozotocin at a dose of 75 mg/kg body weight after 16 hours of fasting. We have found that it is possible to perform intravenous injections in the Chinese hamster (*Boquist* 1969b), but in studies of alloxan and streptozotocin we prefer intraperitoneal administrations since these are easier to perform and the risks for complications are small.

A triphasic pattern of blood glucose and insulin levels has been found in rats and mice after the administration of streptozotocin (*Junod* et al. 1967, *Schein and Bates* 1968, *Schein, Conney and Vernon* 1967). The first hyperglycemic phase has been suggested to be due to mobilization of liver glycogen and the following hypoglycemia to release of insulin from damaged β-cells (*Pitkin and Reynolds* 1970). In guinea pigs streptozotocin has been found to have no effect on the blood glucose level (*Kushner, Lazar, Furman, Lieberman and Leopold* 1969), or to evoke a temporary (*Petersson, Hellerström and Gunnarsson* 1970) or longstanding (*Brosky and Logothetopoulos* 1969) hyperglycemia.

There are only a few reports on the light microscopic changes in streptozotocin-treated animals. The essential findings have been degranulation (*Evans* et al. 1965, *Arison* et al. 1967), or rapid and permanent necrosis of the β-cells (*Junod* et al. 1967). In streptozotocin-treated Chinese hamsters *Richter, Loge and Losert* (1971) have reported that ballooning degeneration and leucocytic infiltration are prominent features in the β-cells.

The initial degenerative changes observed in the light and electron microscopes in the Chinese hamster were similar to those seen after alloxan administration in this species (*Boquist* 1968a and b). Pyknosis, vacuolization, and necrosis of β-cells were evident. We believe that the β-cytotoxic action plays the major role in the development of streptozotocin diabetes in the Chinese hamster. Any obvious leucocytic reaction was not observed during the first day after alloxan injection, but lymphocytic infiltrates occurred around some islets. In contrast to the findings in alloxan-treated hamsters, there were also degenerative alterations in a few α_2-cells in those given streptozotocin. The lesions in these cells do not seem to have been reported in other studies of streptozotocin-diabetic animals. The fact that degeneration and even necrosis occurred in some α_2-cells already 6 hours after the injection of streptozotocin, indicates that these cells may have been subjected to a direct damaging effect of streptozotocin. Aggregates of secretory granules in the β-cells of the type observed in the present study, have also been found in Chinese hamsters with spontaneous diabetes mellitus (*Boquist* 1969a). In only slightly or moderately damaged β-cells the secretory granules often exhibited structural variations of the granule core but a persisting surrounding membrane. The finding of this change, as well as that of aggregates of secretory granules and only slight degranulation may give morphological support to the opinion that streptozotocin exerts a direct and immediate inhibitory effect on insulin secretion in the β-cells (*Golden, Baird, Malaisse, Malaisse-Lagae and Walker* 1971).

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STREPTOZOTOCIN-DIABETES IN THE CHINESE HAMSTER

Volumetric Quantitation of the Pancreatic Islets and Inhibition of Diabetes with Nicotinamide.

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Running title: Streptozotocin-Diabetes in the Chinese hamster.

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SUMMARY

A relationship was found between the dose of intraperitoneally injected streptozotocin and the degree of blood glucose elevation. In an investigation of the long-term effect of 200 mg streptozotocin/ /kg body weight administered intraperitoneally in Chinese hamsters, about 50% of the injected animals died within the experimental period of 3 months in a diabetic state with hyperglycemia and glucosuria. The maximal mortality was noted between the first and second week. The diabetic animals showed a biphasic blood glucose curve; there was a marked hyperglycemic phase one day after injection, followed by a decrease in blood glucose to moderately elevated values which persisted to the end of the experimental period with no signs of returning to normal.

Marked islet atrophy ($p < 0.001$) and a decrease in mean islet diameter ($p < 0.05$) was found in the diabetic animals as compared with normal controls one month after injection.

Pretreatment with 500 mg nicotinamide/kg body weight intraperitoneally 10 minutes before the injection of streptozotocin completely inhibited the diabetogenic effect of the latter drug.

The results show that in the Chinese hamster injection of streptozotocin causes a permanent diabetic state different from alloxan diabetes, with no signs of restitution, similar to spontaneously occurring hereditary diabetes in Chinese hamsters and human diabetes mellitus.

Key words: Chinese hamster - Streptozotocin - Nicotinamide - Hyperglycemia - Pancreatic Islet Volume and Diameter

INTRODUCTION

In the Chinese hamster spontaneous hereditary diabetes mellitus can occur (Meier and Yerganian, 1959) which supposedly is of pancreatic origin. It is irreversible (Boquist, 1969a; Carpenter, Gerritsen, Dulin, and Lazarow, 1967; Carpenter, Gerritsen, Dulin, and Lazarow, 1970; Chang and Schneider, 1970; Gerritsen and Dulin, 1967; Yerganian, 1964) and is considered to be very similar to human diabetes (Renold and Burr, 1970).

Injection of alloxan in non-diabetic Chinese hamsters causes a transitory diabetic state with restitution to normal conditions within a few weeks, both morphologically and functionally (Boquist, 1968a and b). Thus there is a fundamental difference between spontaneous hereditary diabetes and alloxan diabetes in the Chinese hamster.

In the last decade streptozotocin has been widely used for studying experimental diabetes in laboratory rodents (Rerup, 1970; Rudas, 1972), including the Chinese hamster (Losert, Rilke, Loge, Richter, 1971; Richter, Loge, Losert, 1971; Wilander and Boquist, 1972).

In the present investigation the effect of nicotinamide on the induction of streptozotocin diabetes was studied in Chinese hamsters and, further, the long-term effect of streptozotocin in these animals. The purpose of the latter was to allow comparison with alloxan induced and spontaneous hereditary diabetes in the same species, in the first place with regard to blood glucose and the possibility of reversal. As far as I know no investigation of this kind has hitherto been performed.

MATERIAL AND METHODS

Adult, non-diabetic Chinese hamsters of both sexes were used. They were 4-12 months old and weighed 18-38 gm and were selected at random from litters in which no cases of overt diabetes had occurred for more than 6 generations. Before the experiment it was checked that the animals were free from glucosuria and hyperglycemia. They were fed a convential diet for laboratory rodents and given tap water ad libitum.

In a first experiment streptozotocin, (gift from the Upjohn Company, Kalamazoo, Michigan) was administered intraperitoneally to 21 non-fasting Chinese hamsters 4-5 months old in a dose of 100, 150 or 200 mg/kg body weight. Immediately before use the streptozotocin was dissolved in a citrate phosphate buffer at pH 4.0 in a concentration of 2%. Blood samples were taken by the orbital bleeding technique one day after injection and the relationship between the dose of injected streptozotocin and the degree of blood glucose elevation was studied. Blood glucose determinations were performed by the glucose oxidase method (AB Kabi, Stockholm, Sweden).

In a second experiment 43 animals 8-12 months old were used. Twelve non-fasting animals were injected intraperitoneally with streptozotocin 200 mg/kg body weight. Sixteen animals were given a corresponding volume of plain buffer solution. Eight animals were given nicotinamide (AB Kabi, Stockholm, Sweden) in a dose of 500 mg/kg body weight intraperitoneally 10 minutes before the injection of streptozotocin. In seven control animals the same dose of nicotinamide was injected but no streptozotocin was given. The nicotinamide was dissolved in distilled water in a concentration of 10% immediately before use and was administered intraperitoneally. Blood samples

were taken in non-fasting animals one day after injection.

In a third experiment 64 animals 5-12 months old were used. In 48 non-fasting animals 200 mg streptozotocin/kg body weight was injected intraperitoneally. Sixteen control animals were given a corresponding volume of plain buffer solution. Blood samples were taken 1, 3, 6, 13, 20, 27, 55, and 83 days after the injection and blood glucose determinations were performed as described above. The presence of glucosuria was determined by means of clinistix (Ames Co. London, England) and of ketonuria by ketostix (Ames Co. London, England). In eight streptozotocin injected animals no significant blood glucose elevation (< 140.0 mg/100 ml, M + SE x t 0.05 in normal controls) was recorded after one day and these animals were excluded. Fifteen animals died during the experimental period. The rest were sacrificed 1, 2 and 3 months after the injection of streptozotocin. In 10 streptozotocin injected animals 8-10 months old killed after one month the whole pancreas was carefully dissected, fixed in Bouin's fluid, weighed (Mettler K7), dehydrated, paraffin embedded and serially sectioned into alternately 4 x 7 µ and 7 x 4 µ sections. Six non-diabetic control animals of the same age were subjected to the same procedure. The 7µ thick sections were stained with van Gieson's stain and the pancreatic islet areas in the sections were measured planimetrically at a magnificaiton of x 400 (the distance between the sections was 224 µ). The 4 µ sections were used for light microscopic examination after special stainings and these results are reported elsewhere (Wilander, 1973). The total islet volume in each experimental and control animal, as well as the mean islet diameter, was calculated by conventional methods (Hellman, 1959).

In eight experimental animals and eight normal controls the total body weight was measured on a Mettler K7 balance before and 27 days after the start of the experiment.

RESULTS

Dose and Blood Glucose

The effects on the blood glucose of intraperitoneally injected streptozotocin in doses of 100, 150 and 200 mg/kg body weight are shown in Fig. 1. A direct relationship was found between dose and blood glucose response. At a dose of 200 mg/kg body weight all animals were hyperglycemic one day after injection.

Nicotinamide

Treatment with 500 mg nicotinamide/kg body weight 10 minutes before the injection of 200 mg streptozotocin/kg body weight completely inhibited the diabetogenic effect of the latter drug in all animals (Table I). There was a significant difference in blood glucose between the streptozotocin and nicotinamide + streptozotocin injected animals ($p < 0.001$). No significant difference in blood glucose was noted between the buffer, nicotinamide and nicotinamide + streptozotocin injected animals ($p > 0.1$).

Mortality

The mortality in the Chinese hamsters injected with 200 mg streptozotocin/kg body weight was about 30% during the first month (Fig. 2) and about 50% at the end of the experimental period. During the first month the curve was sigmoid and quite steep between the first and second week.

Blood Glucose Variations

The animals that died during the experimental period showed moderate or marked hyperglycemia (about 500 mg/100 ml) up to the point of death, with no signs of a decrease in blood glucose. The blood glucose variations during the first month in the streptozotocin injected animals killed after one and two months are shown in Fig. 3. The individual blood glucose values exhibited large variations but there was a marked hyperglycemic peak in all animals after one day (346.8 ± 35.3 mg/100 ml M ± SEM). The blood glucose then decreased on the third day (194.0 ± 21.1 mg/100 ml), and in the following days and months it was moderately elevated (about 200 mg/100 ml). In some animals normal values were regained on the third day after the initial hyperglycemic peak, and persisted to the end of the observation period. From the third day to the end of the observation period there were no signs of a decrease in blood glucose towards normal values in the diabetic animals. Since 50% of the diabetic animals, those with the highest blood glucose values, had died after three months, the blood glucose in the living animals (129.2 ± 10.0 mg/100 ml) almost reached the level of that in the buffer injected animals at that time.

Glucosuria

All diabetic animals with moderate or marked hyperglycemia up to the end of the experimental period showed glucosuria. The streptozotocin injected animals with a transient hyperglycemic peak one day after injection also showed glucosuria that often persisted in spite of normal blood glucose values. These findings were more pronounced at the end of the observation period. No glucosuria

was found in animals injected with plain buffer solution. In animals with marked hyperglycemia ketonuria was sometimes observed.

Pancreatic Islet Volume and Diameter

The results of calculation of the total pancreatic islet volume in the streptozotocin injected animals killed after one month and in normal controls are given in Table II. There was a significant difference in this volume between the two groups of animals ($p < 0.001$). The mean pancreatic islet diameters in these same animals are also given in Table II. The islets in the experimental animals were significally smaller than in the normal controls ($p < 0.05$).

Body and Pancreatic Weight

The total pancreatic weight was smaller in the diabetic animals than in normal controls ($p < 0.05$), as seen in Table II. This may be due at least partly to loss in body weight in the experimental animals from 24.4 gm to 21.1 gm. In the control group no weight loss was observed.

DISCUSSION

A relationship was found in Chinese hamsters between the dose of intraperitoneally administered streptozotocin and the diabetogenic response. A dose of 200 mg/kg body weight resulted in blood glucose elevation in most animals. Chinese hamsters appear to be as sensitive to streptozotocin as mice, in which animals the corresponding dose has been reported to produce diabetes in most

cases (Rerup and Tarding, 1969; Schein and Bates, 1968; Schein, Cooney, and Vernon, 1967). The dose of alloxan giving blood glucose variations and histological changes in most Chinese hamsters is about 250 mg/kg body weight (Boquist, 1968a). When consideration is taken of the molecular weights of alloxan (160.08) and streptozotocin (265.00), Chinese hamsters seem to be more suspectible to the diabetogenic effect of the latter drug. The approximate L.D. 50 of alloxan and streptozotocin at an observation time of 48 hours is 380 and 400 mg/kg body weight, respectively, in non-fasting animals (Boquist, 1968a; Wilander and Boquist, 1972). Thus streptozotocin seems to be the most toxic of these two diabetogenic substances. In the present study, covering a longer period, almost 50% of the animals died mainly between the first and second week. This has been considered to be due at least partly to the diabetic state per se and not only to a toxic effect of streptozotocin.

The short-term effect of streptozotocin on the blood glucose in Chinese hamsters has been reported earlier (Losert, Rilke, Loge, and Richter, 1971; Wilander and Boquist, 1972). In the present study persistent hyperglycemia was noted in most animals after the injection of streptozotocin in Chinese hamsters, with a marked peak one day after injection, or in some animals a transitory hyperglycemia with a maximum one day after injection and a rapid return to a normal blood glucose level. These results are in contrast to the findings after injection of alloxan in Chinese hamsters, when the hyperglycemia persists for about 8-9 days with a maximum after 4 days. The effect of alloxan on the blood glucose in Chinese hamsters conforms with that in guinea pigs (Johnson, 1950), rats (Lazarow, 1952) and mice (Rerup and Tarding, 1969). Streptozotocin injection in mice (Brosky and Logothetopoulos, 1969; Rerup and Tarding, 1969) and rats (Hoftiezer and Carpenter, 1973;

Junod, Lambert, Stauffacher, and Renold, 1969; Steiner, Oelz, Zahnd, and Froesch, 1970) results in prolonged hyperglycemia with no signs of restitution. On the other hand, guinea pigs injected with streptozotocin show no hyperglycemia (Kushner, Lazar, Furman, Lieberman, and Leopold, 1969) or only a temporary hyperglycemia with a return to normal or sub-normal blood glucose levels despite continued glucosuria. This might be explained by tubular necrosis in the kidneys (Petersson, Hellerström, and Gunnarsson, 1970). Streptozotocin injected guinea pigs without reversion of the diabetic syndrome have also been observed, however, (Brosky and Logothetopoulos, 1969). Thus the effect of streptozotocin in Chinese hamsters seems to conform best with observations made on mice and rats in that no obvious restitution takes place.

The marked increase in blood sugar one day after injection, followed by a decrease, may not only be the result of necrosis of the insulin producing β-cells, as it has been reported (Golden, Baird, Malaisse, Malaisse-Lagae, and Walker, 1971) that streptozotocin causes a dose related inhibition of glucose induced insulin secretion. If this effect is transitory a return from high to lower blood glucose levels might be expected. Further, the decrease in blood glucose after one day appears too early to be explained by islet cell regeneration.

It is well known that in certain animal species, such as mice, guinea pigs and rabbits, but also in infants of diabetic mothers (Cavallero, 1947; Friedman and Marble, 1941; Hellman and Hellerström, 1968; Lazarow, 1952; Lazarus and Volk, 1952 and 1958; Patent and Alfert, 1967), hyperglycemia seems to constitute a stimulus to β-cell proliferation and neoformation. The significant atrophy of the pancreatic islet tissue one month after the injection of

streptozotocin in Chinese hamsters indicates that no obvious restitution of the islet tissue occurred. It has been claimed (Logothetopoulos and Brosky, 1968) that the pancreatic β-cells have a limited potential for sustained regeneration. Thus it is possible that in the present investigation this limit was reached and was insufficient for restoration of the islet tissue to normal. Supporting this theory is the persistent hyperglycemia in most animals. Streptozotocin has been shown to inhibit the progression of cells into mitosis (Bhuyan, 1970). This may also have some significance for the obtained results.

The diabetogenic effect of streptozotocin is due to its marked β-cytotoxic activity (Junod, Lambert, Orci, Pictet, Gomez, and Renold, 1967; Rakieten, Rakieten, and Nadkarni, 1963). Thus it was expected that the diabetic animals might have a smaller pancreatic islet volume and a smaller islet diameter than normal controls. This was in fact found to be true in the streptozotocin injected Chinese hamsters. The same findings have been made in human juvenile diabetes mellitus (Gepts, 1965; Maclean and Ogilvie, 1955 and 1959) and in Chinese hamsters with spontaneously occurring hereditary diabetes (Carpenter, et al., 1967; Meier and Yerganian, 1957).

As with alloxan (Lazarow, Liambies, and Tausch, 1950), it has been reported that the effect of streptozotocin can be prevented by injection of nicotinamide (Dulin and Wyse, 1969; Schein and Bates, 1968; Schein, Cooney, and Vernon, 1967; Stauffacher, Burr, Gutzeit, Beaven, Veleminsky, and Renold, 1970). This finding was also made in the present investigation on Chinese hamsters. In contrast to alloxan, however, injection of nicotinic acid gives no such protection (Dulin and Wyse, 1969; Schein, Cooney, and

Vernon, 1967; Stauffacher, et al., 1970). Both nicotinamide and nicotinic acid are precursors of nicotinamide adenine dinucleotide (NAD) (Ichiyama, Nakamura, and Nishizuka, 1969). Administration of streptozotocin results in a depletion of the NAD content of the liver and nicotinamide is capable of preventing such depletion (Schein, Cooney, and Vernon, 1967; Schein and Loftus, 1968). In a recent investigation by Ho and Hashim (1972) a decrease in the pancreatic islet NAD content was found after administration of diabetogenic doses of streptozotocin. These authors conclude that the β-cell deterioration in streptozotocin induced diabetes may be causally related to depletion of cellular NAD.

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TABLE I. Inhibition of streptozotocin diabetes with nicotinamide in Chinese hamsters.

Treatment	Blood glucose (mg/100 ml) one day after injection M ± SEM
Buffer solution	[illegible]
Streptozotocin 200 mg/kg	[illegible]
Nicotinamide 500 mg/kg	[illegible]
Nicotinamide 500 mg/kg + streptozotocin 200 mg/kg	[illegible]

TABLE II. Blood glucose, pancreatic weight, total islet volume and mean islet diameter in streptozotocin injected Chinese hamsters 1 month after injection and in normal controls.

Animals	Blood glucose (mg/100 ml) M ± SEM	Islet volume (mm^3) M ± SEM	Mean islet diam. (μ) M ± SEM	Pancreatic weight (gm) M ± SEM
Streptozotocin--diabetic animals	201.3 ± 48.3 n = 10	0.0437 ± 0.0049 n = 10	93.6 ± 0.5 n = 10	0.1406 ± 0.042 n = 6
Normal controls	107.1 ± 6.7 n = 16	0.1952 ± 0.0323 n = 6	110.7 ± 0.6 n = 6	0.1564 ± 0.041 n = 6

Fig. 1 Relationship between the dose of intraperitoneally injected streptozotocin and the degree of blood glucose elevation (M $\pm$ SEM).

Fig. 2. Cumulated mortality in per cent in streptozotocin-diabetic Chinese hamsters within one month after injection.

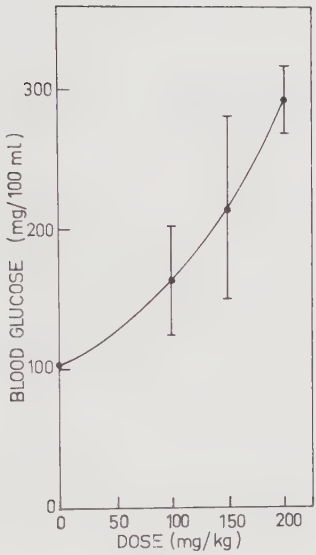
BLOOD GLUCOSE (mg/100 ml)
300
200
100
0
0
50
100
150
200
DOSE (mg/kg)

Fig. 1.

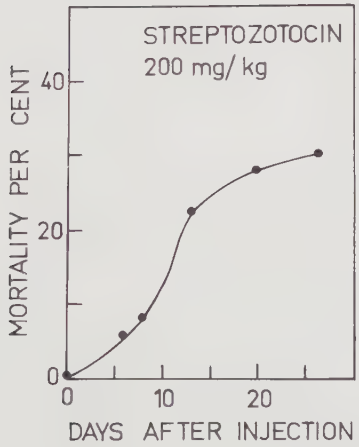
STREPTOZOTOCIN
200 mg/ kg
MORTALITY PER CENT
40
20
0
0
10
20
DAYS AFTER INJECTION

Fig. 2.

Fig. 3. Blood glucose variations ($\bar{M} \pm$ SEM) in 12 streptozotocin-diabetic Chinese hamsters within one month after injection and in 16 normal controls.

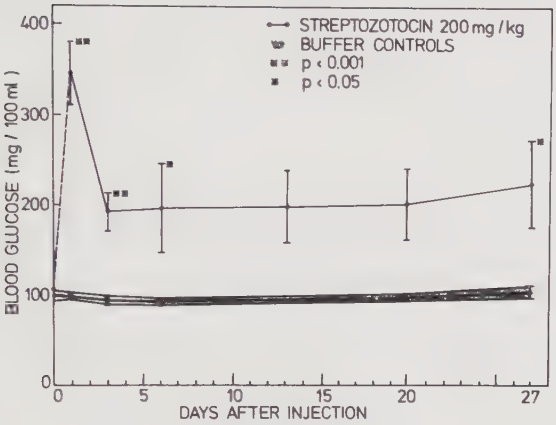
STREPTOZOTOCIN 200 mg / kg
BUFFER CONTROLS
p < 0.001
p < 0.05
BLOOD GLUCOSE (mg / 100 ml)
400
300
200
100
0
0
5
10
15
20
27
DAYS AFTER INJECTION

Fig. 3.

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STREPTOZOTOCIN-DIABETES IN THE CHINESE HAMSTER

Long-term effects on the light microscopic morphology of the pancreatic islet tissue, liver and kidney.

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Running title: Streptozotocin-diabetes in the Chinese hamster.

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SUMMARY

The long-term effects of 200 mg streptozotocin/kg body weight on the light microscopic morphology of the pancreatic islet tissue, liver and kidney were studied in Chinese hamsters. One day after injection of streptozotocin necrotic cell debris or marked degenerative changes were seen in the pancreatic islets. Both β-, α_2- and α_1-cells seemed to be affected. During the first weeks, in particular, signs of regeneration and neoformation of islet tissue were evident in the pancreatic tissue. Labelling with ^{3}H-thymidine revealed an increased incorporation of this isotope in islets of diabetic animals. Tubular structures and proliferating buds consisting mostly of "clear cells" were seen. Aggregates of "clear cells" were also observed in islets with degenerative changes and in exocrine pancreas, with no obvious relationship to islets. After one week, up to about one month, large islets containing hypertrophied cells with a large nucleus and a prominent nucleolus were found. Hydropic degeneration and even necrosis sometimes occurred in these islets. The regenerative activity as well as the hypertrophic islets disappeared at the end of the observation period (3 months) at which time relatively small islets with degranulated β-cells and irregularly distributed α_2- and α_1-cells with variable staining properties and nuclear sizes were seen. In the liver fatty vacuolization occurred initially. At the end of the observation period liver cell polymorphism and proliferation of portal tubular structures were evident.

In the kidneys tubular dilatation and tubular epithelial degeneration were observed, mainly in the later part of the observation period.

Key words: Chinese Hamster - Streptozotocin - Pancreatic Islet Cell Degeneration and Regeneration - Liver - Kidney

INTRODUCTION

The effect of the diabetogenic substance streptozotocin on the blood glucose and the volume and diameter of the pancreatic islets in Chinese hamsters has been described in a previous report (Wilander 1974), as well as light and electron microscopic changes in the pancreatic islet tissue in the first 24 hours after injection (Wilander and Boquist 1972). It has been shown that streptozotocin exerts a direct cytotoxic effect on the pancreatic islet β-cells and even α_2-cells. The streptozotocin injected animals either show a hyperglycemic phase 24 hours after injection, with return to normal blood glucose values within three days, or in most animals a persistent hyperglycemia with no signs of reversal.

The aim of the present investigation was to study the long-term effect of streptozotocin on the light microscopic morphology of the pancreatic islet tissue, liver and kidneys in Chinese hamsters, mainly for comparison with previously described blood glucose variations (Wilander 1974) as well as with the light microscopic pancreatic islet morphology in alloxan-diabetic (Boquist 1968) and spontaneous hereditary diabetic (Meier and Yerganian 1959; Carpenter et al. 1967 and 1970) animals of the same species.

MATERIAL AND METHODS

Non-diabetic Chinese hamsters of both sexes were used, partly the same animals as have been referred to in earlier reports (Wilander and Boquist 1972; Wilander 1974). They were 4-14 months old, weighed 17-39 gm and were selected at random from litters in which no cases of overt diabetes had occurred for at least six generations. The animals were free from hyperglycemia and glucosuria before the start of the experiment.

One hundred non-fasting Chinese hamsters were injected intraperitoneally with 200 mg streptozotocin/kg body weight. The streptozotocin was dissolved in citrate phosphate buffer at pH 4.0 and a concentration of 2%. Eighteen control animals were given a corresponding volume of plain buffer intraperitoneally.

Five streptozotocin injected animals and two buffer controls were injected with ^{3}H-thymidine (spec. act. 5 Ci/mM) intraperitoneally in a dose of 1 µCi/gm body weight 8 times from the first to the fourth day after injection of streptozotocin.

Blood glucose analyses and tests for urinary glucose were performed by the same methods and at the same time intervals as were used in previous studies (Wilander 1974).

Twenty-five diabetic animals died spontaneously during the experimental period. The rest were sacrificed 1, 2, 3, 4, 6, 10, 11, 13, 20, 27, 55 and 83 days after the injection. The ^{3}H--thymidine injected animals were killed on the fourth day. Specimens from each sacrificed animal were taken from the pancreas, liver and kidneys. Those from the pancreas were fixed in Bouin's fluid, dehydrated, embedded in paraffin, sectioned

in 4 µ thick sections and stained with van Gieson's stain, aldehyde-fuchsin, sometimes counterstaind with Ponceau-fuchsin (Hultquist 1962), chrome alum hematoxylin, toluidine blue (1% pH 7.0), alkaline Congo red (Puchtler et al. 1962) and silver impregnations according to Hellerström and Hellman (1960) and Grimelius (1968). From the ^{3}H-thymidine injected animals sections from the pancreas were taken for a microautoradiographic study using the conventional liquid emulsion method (Ilford K2 diluted 2:1 with redistilled water 45°C). After exposure and development the sections were stained with Ehrlich's hematoxylin. Specimens from the kidneys and liver were fixed in 10% formalin and stained with van Gieson's stain and hematoxylin-eosin. Specimens from the kidneys were also stained with PAS.

RESULTS

Blood glucose: The result of the blood glucose analyses has been described in an earlier report (Wilander 1974).

Pancreatic Islet Tissue

Degenerative changes. Twenty-four hours after the injection of streptozotocin there was almost total destruction of the endocrine pancreas in most animals. No β-, α_2- or α_1-cells could be found. The islets contained only capillaries and a few inflammatory cells surrounded by an amorphous material (Fig. 1), but the changes varied within the same pancreas and between different animals. In some islets the degenerative changes were less pronounced. In these islets the β-cells showed an irregular

pyknotic nucleus and vacuolization, and the staining properties of their cytoplasm varied (Fig. 2). Irregular α_2- and α_1-cells were also seen in these islets, often with only slight granulation. Mitoses were sometimes observed in the few remaining cells of the nearly totally destroyed islets as well as in those with marked degenerative changes. In the buffer injected animals the islet tissue appeared normal (Fig. 3).

Regenerative changes. During the first month but mainly during the first and second weeks there were signs of regeneration and neoformation of islet tissue, with tubular structures either lying within islet tissue or unrelated to it. "Clear cells" could be seen to proliferate from these structures (Fig. 4). Aggregates of "clear cells" were also seen in the pancreatic tissue without obvious association with islet tissue or tubular structures (Fig. 5), as well as in islets with degenerative changes (Fig. 6).

In the diabetic animals islets were relatively rarely found in sections from the pancreas, but in the few islets observed there was a high incorporation of ^{3}H-thymidine after injection of this isotope (Fig. 7). Labelled cells were found both in the centre and in the periphery of the islets, but could not be identified with certainty. In buffer injected animals labelled islet cells were occasionally seen (Fig. 8).

The degree of regeneration of islet tissue varied in different animals. In several animals there were no or only slight signs of neoformation of islet tissue. The regenerative activity declined during the experimental period. At the end of this period no signs of regeneration could be found except for the occasional presence of "clear cells" in the islets.

Small aggregates of lymphocytes were seldom seen in the normal pancreas. These seemed to increase in number in the diabetic animals, mainly in association with tubular structures and "clear cells" (Fig. 4).

Hydropic degeneration. Large islets with hydropic degeneration were found in the pancreatic tissue in streptozotocin treated Chinese hamsters. Islets of this kind were seen in some cases after about one week and in all diabetic animals after one month, after which time they gradually disappeared. They consisted of hypertrophied cells with a large nucleus and a prominent nucleolus (Fig. 9). Especially in their peripheral area the cells often showed marked hydropic degeneration, sometimes obviously resulting in necrosis and fragmentation preceded by pronounced nuclear polymorphism and hyperchromatism. Tubular structures and aggregates of lymphocytes were occasionally seen in association with these islets. The cytoplasm of the endocrine cells in the periphery of these islets failed to stain with silver impregnations but was often slightly positive to Ponceau-fuchsin stain. A few weakly aldehydefuchsin stained cells were seen in the centres. It is possible that many of the endocrine cells with hydropic degeneration in the peripheral areas of the islets were α_2-cells.

Additional findings. On the whole the islets appeared smaller in the diabetic animals than in the normal controls. Further, the nucleus varied in size and often seemed larger in the β-cells as well as in the α_2-cells in the streptozotocin injected animals (Fig. 10). The β-cells were weakly stained. The α_2-cells were irregularly distributed in the islets and showed great variation in staining properties compared with normal controls. Essentially normal α_1-cells were found in the peripheral areas of the islets in the diabetic animals.

No amyloid deposits were observed either in the experimental animals or in the normal controls.

Liver Morphology

Fatty vacuolization was found in the liver in about 50% of the diabetic animals 24 hours after the injection of streptozotocin. It was less pronounced after three days and disappeared during the following days. In a few diabetic animals the liver cells showed nuclear polymorphism and in the portal regions proliferating tubular structures (Fig. 11) after one month, and these observations were made in about half of the cases after three months.

Renal Morphology

No obvious changes in the kidneys were seen 24 hours after the injection of streptozotocin. After three days and during the following days and months tubular dilatation and degenerative changes in the tubular epithelium were observed (Fig. 12), sometimes with small accumulations of glycogen. These changes were more pronounced at the end of the observation period, at which time they were evident in almost all diabetic animals. No glomerular or appreciable tubular necrosis was found. There was also some dilatation of the renal pelvis at the end of the observation period in the diabetic animals.

DISCUSSION

This study confirms the observations made in a previous investigation (Wilander and Boquist 1972) that streptozotocin acts as a

β-cytotoxic substance in Chinese hamsters, causing necrosis or marked degenerative changes in the β-cells with nuclear pyknosis and cytoplasmic vacuolization. Degenerative changes and necrosis were also found in the α_2-cells, findings which have also been made in the rabbit (Lazarus and Shapiro 1972) but in no other laboratory animal hitherto examined. There seems to be a difference in the cytotoxic action of alloxan and streptozotocin in the Chinese hamster, as degenerative changes without obvious necrosis in the β cells, together with damage to α_2-cells have not been reported after administration of alloxan (Boquist 1968).

Tubular structures with proliferating buds of "clear cells" as signs of regeneration and restoration of the pancreatic islet tissue to normal have been described in alloxan diabetic mice (House 1958; Bunnag et al. 1967) and in alloxan diabetic Chinese hamsters (Boquist 1968). In streptozotocin diabetic rats Arison et al. (1967) found only slight morphological signs of regeneration and Steiner et al. (1970) claimed that β-cell regeneration from insular cells or islet neoformation from extrainsular cells played no significant role after streptozotocin injection in the same animal. Logothetopoulos and Brosky (1968) observed significant mitotic activity in the early phase after streptozotocin injection in mice. This activity was much less marked than after injection of alloxan. In the diabetic mutant mouse an increased incorporation of ^{3}H-thymidine in the β-cells has been observed in the prediabetic phase, during the onset of early hyperglycemia and in animals with established hyperglycemia; during the terminal phase of this syndrome, however, labelled endocrine cells were infrequent (Like and Chick 1970). The results obtained in this study are mainly in accordance with observations made in the diabetic mutant mouse and streptozotocin diabetic mice. Although

there were obvious signs of neoformation of islet tissue, especially during the early phase after streptozotocin injection, this seemed to be insufficient to restore the islet tissue to normal. Islet neoformation in streptozotocin diabetic Chinese hamsters seems to take place through mitoses of endocrine islet cells and probably also through cell proliferation from tubular structures. The occurrence of "clear cells" in a period of increased incorporation of labelled thymidine in islet tissue indicates that these cells may participate in islet cell regeneration. The persistent marked islet atrophy which has been observed in streptozotocin diabetic Chinese hamsters (Wilander 1974) is worthy of note since a decrease in islet cell volume and a decrease in β-cell mass also characterize Chinese hamsters with spontaneous hereditary diabetes (Carpenter et al. 1967), suggesting that these alterations are a primary aetiologic mechanism in the production of the diabetic syndromes.

These observations may also be of some importance with respect to the aetiology of human juvenile diabetes mellitus since a decrease in β-cell mass has also been described in this condition (Gepts 1965:, Maclean and Ogilvie 1955 and 1959).

During the first month, and, especially during its later part large islets consisting of endocrine cells with a large nucleus, a prominent nucleolus, varying degrees of vacuolization in the cytoplasm, and also swelling were seen. Necrosis of what at least may have been partly α_2-cells was also found in the periphery of these islets. An increase in nuclear size suggests an increased protein synthetic activity (Hultquist 1962; Oehlert and Schultze 1960; Patent and Alfert 1967), in the present investigation sometimes probably resulting in exhaustion. An increase in mean nuclear

diameter has been reported in β-cells and α_2-cells of streptozotocin diabetic rats (Steiner et al. 1970) and in β- and α_1-cells of streptozotocin-treated guinea pigs Petersson et al. (1970). Katsilambros et al. (1970) found elevated levels of serum glucagon in rats after streptozotocin injection. Likewise, Unger et al. (1970) demonstrated strikingly elevated plasma glucagon levels in diabetic patients with severe keto-acidosis. The mechanism underlying the increase in α_2-cell activity remains unclear.

The fatty metamorphosis of liver cells observed in several Chinese hamsters 24 hours after injection of streptozotocin has not been reported in other laboratory rodents after streptozotocin injection. The changes disappeared after a few days and may have been a result of a cytotoxic effect. At the end of the observation period an increased number of tubular structures were seen in the portal region of the liver parenchyma. These were suggestive of incipient neoplastic transformation. Streptozotocin is known to possess carcinogenic properties and is reported to cause renal and pancreatic tumours (Arison and Feudal 1967; Rakieten et al. 1968; Steiner et al. 1970). No renal or pancreatic tumours were observed in the Chinese hamsters, however, after streptozotocin injection.

Marked parenchymatous degeneration with atrophy of tubular cells and secondary cystic dilatation of the lumina of the kidney tubulus has been observed in duct-ligated and alloxan treated rats (Vargas et al. 1970; Edström 1972). No appreciable changes in the kidneys of alloxan treated Chinese hamsters have been reported (Boquist 1968). In Chinese hamsters with spontaneous hereditary diabetes, hydronephrosis as well as tubular dilatation with tubular epithelial degeneration and intercapillary glomerulo-

sclerosis have been described (Meier and Yerganian 1959). The renal changes observed after streptozotocin injection in Chinese hamsters most resemble those found in alloxan-diabetic and duct--ligated rats. The cause of these changes is not clear but the diabetic state may play a role by interfering with normal tubular regeneration (Vargas et al. 1970). No relevant answers to this question are offered by the present experimental results. It may be noted, however, that no diabetic glomerulosclerosis appeared in the streptozotocin-injected Chinese hamsters.

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Fig. 1. Pancreatic islet in a Chinese hamster 24 hours after the injection of streptozotocin. There is total destruction of the endocrine cells. The islet contains amorphous material, capillaries and a few inflammatory cells. Aldehyde fuchsin, x 400.

Fig. 2. Pancreatic islet in a Chinese hamster 3 days after injection of streptozotocin. Degenerative endocrine cells with nuclear pyknosis and cytoplasmic vacuolization are seen. Aldehyde fuchsin, x 400.

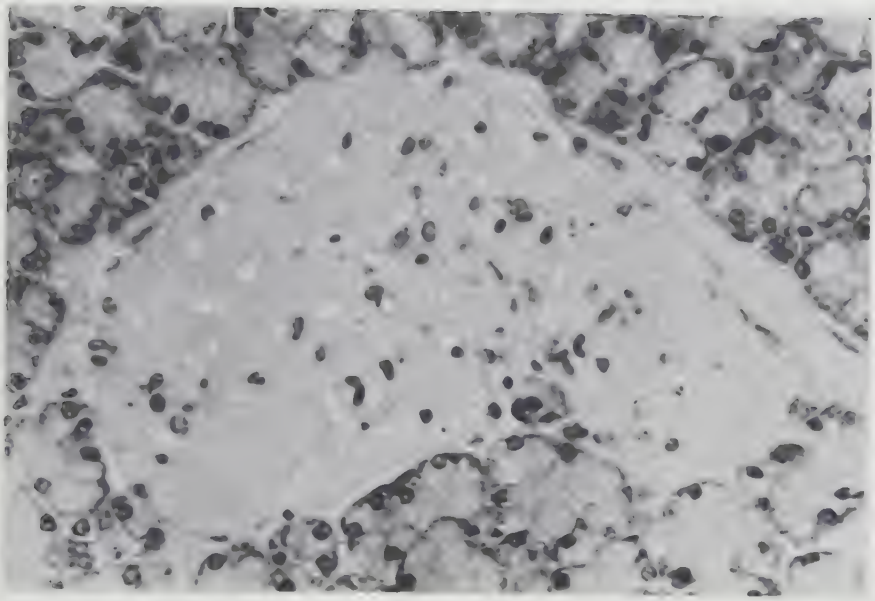

Fig. 1.

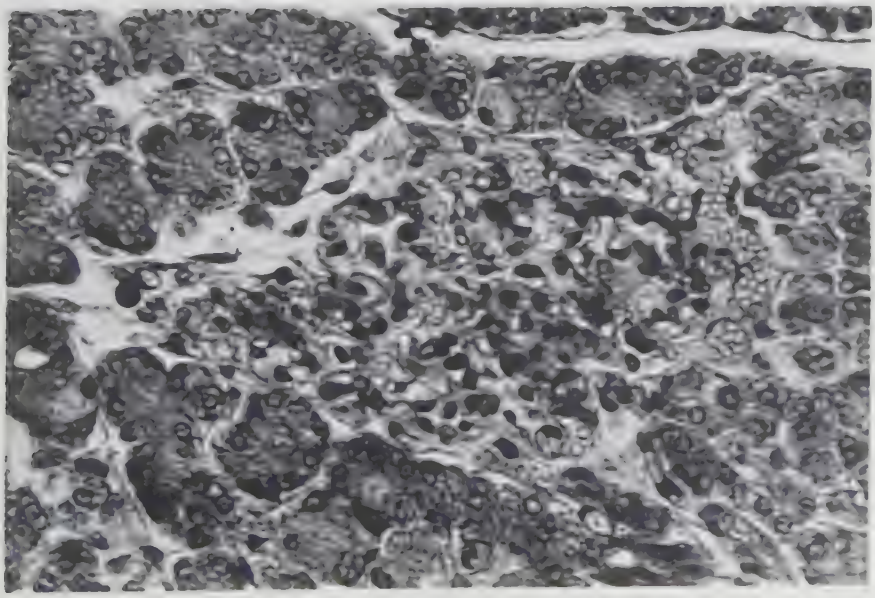

Fig. 2.

Fig. 3. Pancreatic islet in a normal control. Aldehyde fuchsin, x 400.

Fig. 4. Pancreatic tissue in a Chinese hamster 11 days after injection of streptozotocin. Tubular structures with proliferating buds containing "clear cells" are seen. The tubular structures are surrounded by mononuclear inflammatory cells. Aldehyde fuchsin, x 640.

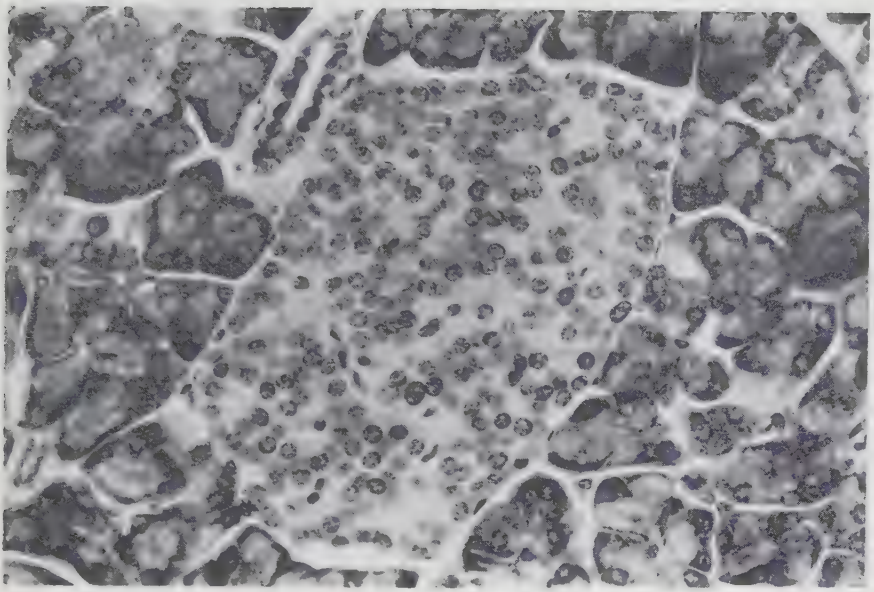

Fig. 3

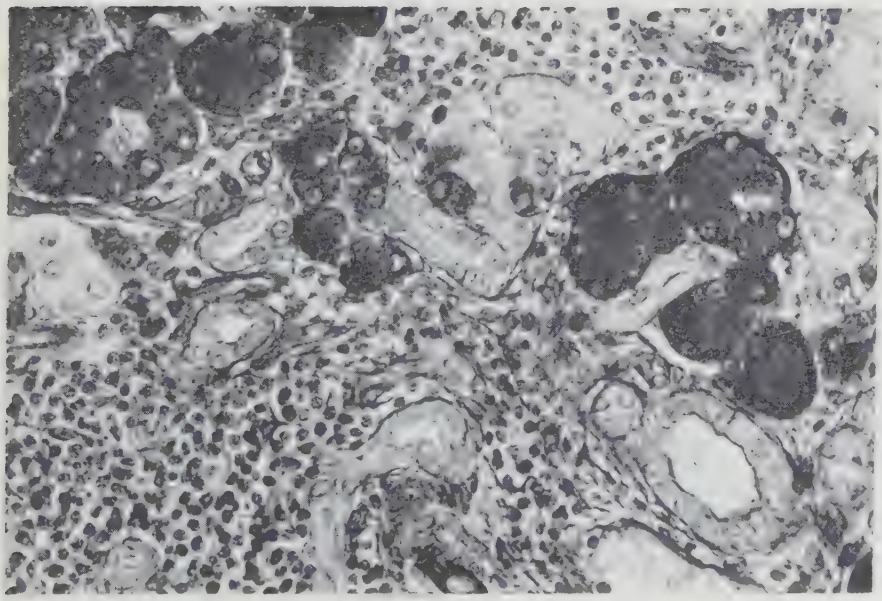

Fig. 4.

Fig. 5. Pancreatic tissue in a Chinese hamster 11 days after injection of streptozotocin. In the exocrine pancreas there is a cluster of "clear cells". These show no clear relationship to tubular structures or mature islets. Aldehyde fuchsin, x 640.

Fig. 6. Pancreatic islet in a Chinese hamster 5 days after injection of streptozotocin. "Clear cells" intermingle with the degenerative endocrine cells. Aldehyde fuchsin, x 400.

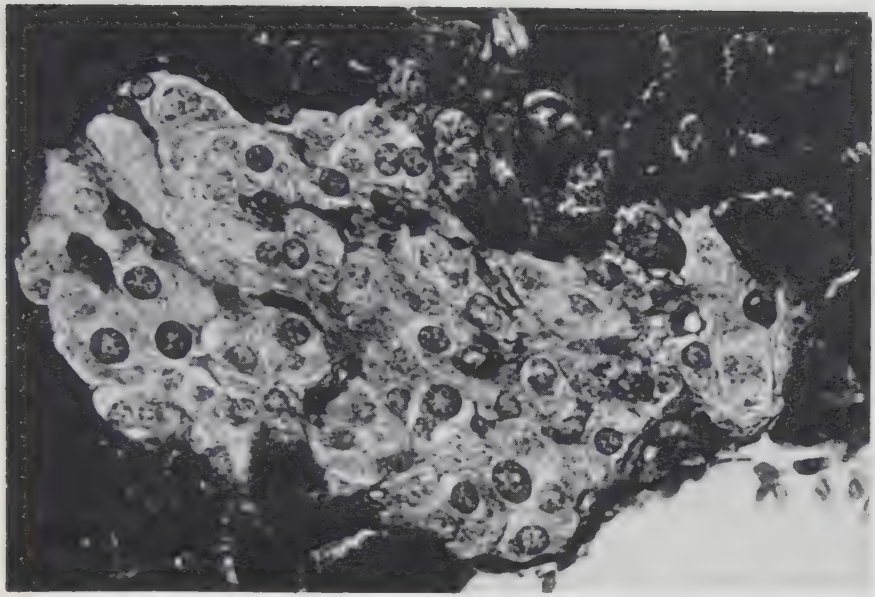

Fig. 5.

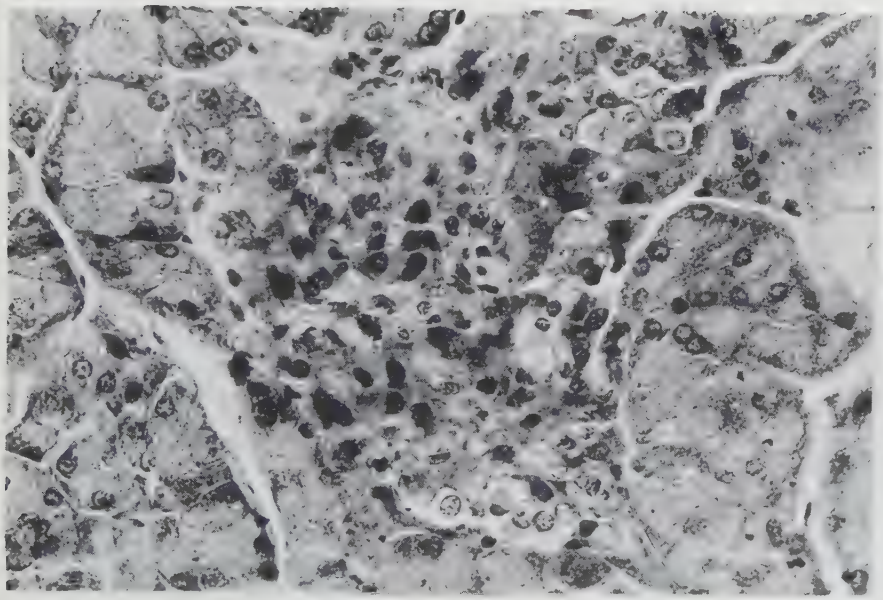

Fig. 6

Fig. 7. Microautoradiograph of ^{3}H-thymidine injected pancreatic islet in a Chinese hamster 4 days after injection of streptozotocin, exhibiting an increase in labelled cells. Ehrlich's haematoxylin, x 400.

Fig. 8. Microautoradiograph of ^{3}H-thymidine injected pancreatic islet in a normal control, showing a few labelled cells in the periphery of the islet. Ehrlich's haematoxylin, x 400.

Fig. 9. A large pancreatic islet in a Chinese hamster 11 days after injection of streptozotocin. It consists of hypertrophied cells with a large nucleus, a prominent nucleolus and hydropic degeneration in the periphery. Aldehyde fuchsin, x 400.

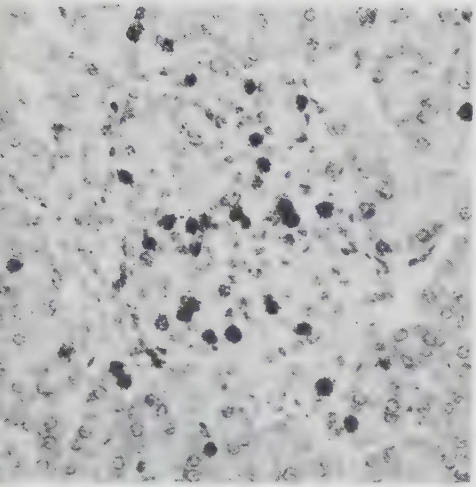

Fig. 7

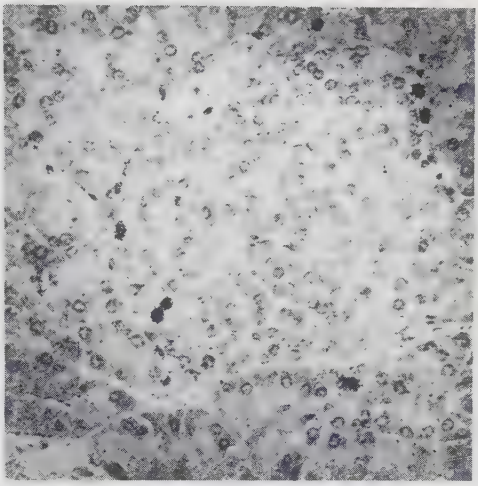

Fig. 8.

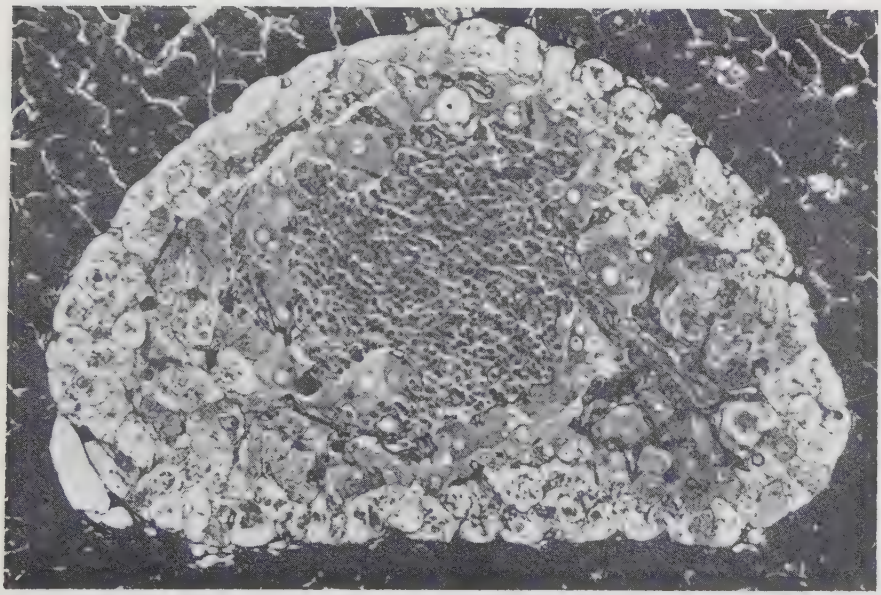

Fig. 9.

Fig.10. Pancreatic islet in a Chinese hamster 55 days after injection of streptozotocin. The islet cells show varied nuclear sizes and often a distinct nucleolus as signs of increased activity. Van Gieson stain, x 640.

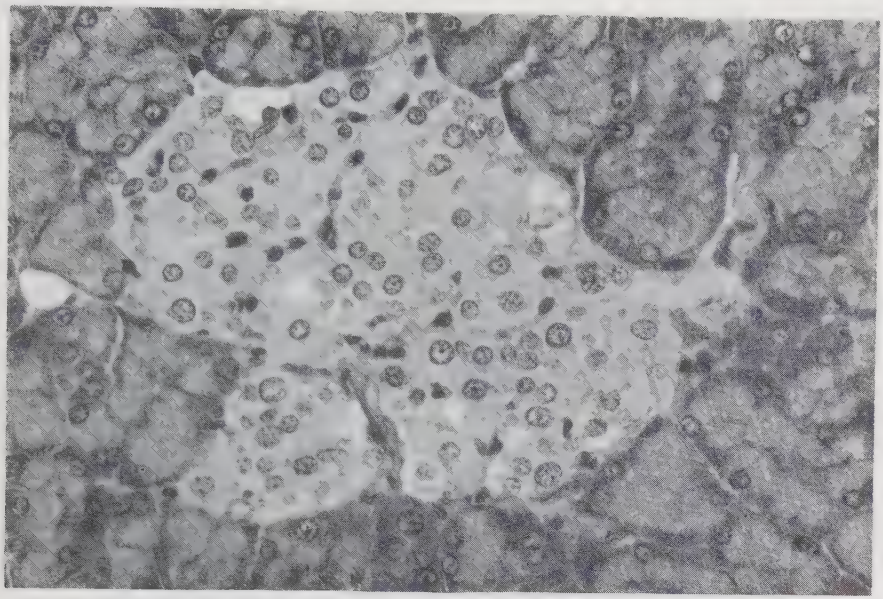

Fig. 10.

Fig.11. Liver tissue of a Chinese hamster 83 days after injection of streptozotocin, showing liver cell polymorphism and proliferating relatively regular tubular structures in the portal regions. Van Gieson stain; Haematoxylin-eosin, x 160.

Fig.12. Renal tissue of a Chinese hamster 83 days after injection of streptozotocin. Degenerative changes in the tubular epithelium and tubular dilatation are seen. Haematoxylin-eosin, x 160.

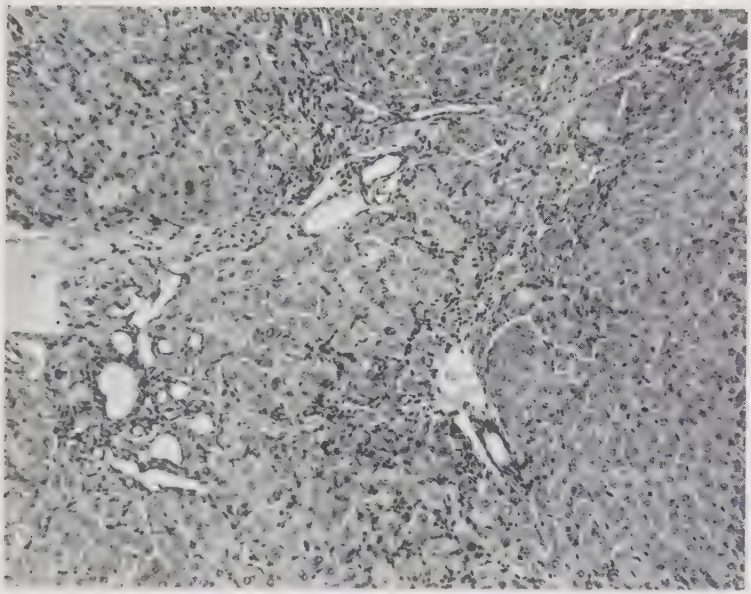

Fig. 11

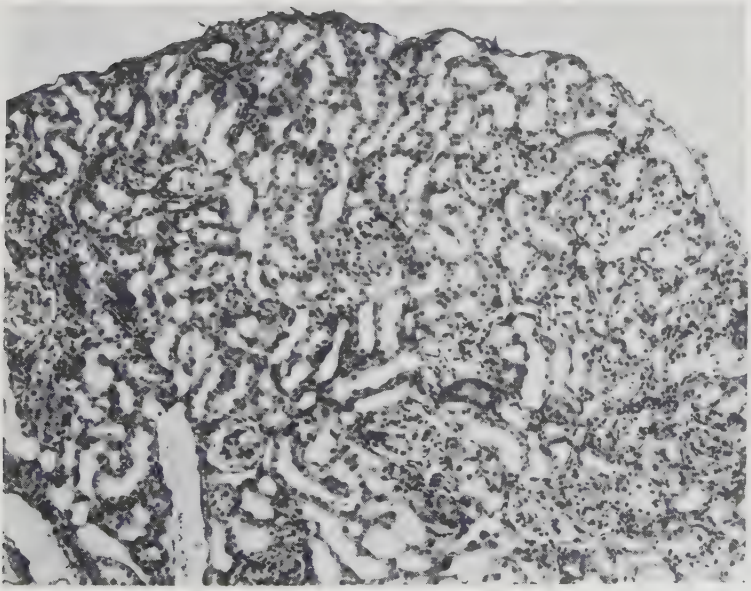

Fig. 12.

DIABETOGENIC EFFECTS OF N-NITROSOMETHYLUREA IN THE CHINESE HAMSTER

by

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Running title: Diabetogenic effects of N-nitrosomethylurea.

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SUMMARY

To evaluate the role of the glucose residue of the diabetogenic substance streptozotocin, the effect of its aglucone derivate N-nitrosomethylurea was tested in Chinese hamsters. At a dose of 50 mg/kg body weight of N-nitrosomethylurea a triphasic blood glucose curve was recorded with an initial hyperglycemic peak after 3 hours followed by a decrease at 6 hours. After 24 hours and during the following days the values were moderately elevated. There was a high mortality in the diabetic animals, about 80% of them dying within one week. The approximate L.D. 50 of N-nitroso-methylurea injected intraperitoneally in non-fasting adult animals was about 125 mg/kg body weight at an observation time of 48 hours.

On light microscopy degenerative changes with nuclear pyknosis were seen after 3 hours in the pancreatic islet cells, followed by cellular disintegration. Both β-, α_2- and α_1-cells were obviously affected. Pretreatment with 500 mg nicotinamide/kg body weight given intraperitoneally 10 minutes before the injection of N-nitroso-methylurea inhibited the hyperglycemia and seemed to prevent the injurious effects of N-nitrosomethylurea on the islet cells. The results show that the glucose residue of the streptozotocin molecule is not necessary for the induction of diabetes in Chinese hamsters, but it seems to increase the selectivity of the toxic effects for the islet cells. This is a clear advantage in studies of experimental diabetes, especially when longer observation periods are desired.

Key words: Experimental Diabetes - Chinese Hamster - N-Nitroso-methylurea - Pancreatic Islet Cell Degeneration - Hyperglycemia

INTRODUCTION

Streptozotocin is a substance with diabetogenic properties, which were first reported by Rakieten, Rakieten, and Nadkarni[1]. Beside its β-cytotoxic effect, which is well documented in laboratory rodents,[2-3] it has also been shown to damage the pancreatic α_2--cells in rabbits[4] and Chinese hamsters[5]. According to Herr, Jahnke, and Argoudelis[6] streptozotocin consists of N-nitrosomethylurea linked to position C_2 of 2-deoxy-D-glucose. The N-nitrosomethylurea moiety of streptozotocin, which can be regarded as the aglucone of this agent, has previously been claimed to lack diabetogenic activity[7] but has recently been shown to damage islet β-cells in mice[8]. Both streptozotocin and N-nitrosomethylurea cause a depression of the nicotinamide adenine dinucleotide (NAD) concentration in pancreatic islet tissue. This effect is thought to be primarily involved in the β-cytotoxic action of the drug, since both the NAD depletion and the β-cell injuries can be prevented by large doses of nicotinamide[8,9]. As similar metabolic effects were observed in the pancreatic islet tissue after injection of streptozotocin and N-nitrosomethylurea, it was decided to study the influence of the latter substance on the light microscopic morphology of the pancreatic islets and on the blood glucose concentration in the Chinese hamster, an animal species in which the diabetogenic and general toxic effects of streptozotocin are well documented[5,10-11].

MATERIAL AND METHODS

Adult non-diabetic Chinese hamsters of both sexes were used. They were selected at random from families in which no case of overt diabetes had occurred for more than six generations. Before the

experiment it was checked that the animals were free from hyperglycemia and glucosuria. The hamsters had free access to a conventional diet for laboratory rodents and were given tap water ad libitum.

In one experiment 48 non-fasting Chinese hamsters were used. N-nitrosomethylurea (Pfaltz and Bauer, Flushing, New York, USA) was injected intraperitoneally in a dose of 50, 100, 150 or 200 mg/kg body weight. Immediately before use the N-nitrosomethylurea was dissolved in a citrate phosphate buffer, pH 4.0, at a concentration of 2% (w/v). After an observation time of 48 hours the approximate L.D. 50 was estimated. Specimens were taken from the pancreas, liver and kidneys for light microscopic examination in animals dying spontaneously. In animals still alive two days after the injection of the drug blood glucose analyses were performed. Blood samples were taken by the orbital bleeding technique under ether anesthesia and blood glucose was determined by a glucose-oxidase method with the aid of a commercially available kit (AB Kabi, Stockholm, Sweden).

In another experiment 59 non-fasting Chinese hamsters were used. Thirty-one animals were injected intraperitoneally with 50 mg/kg body weight of a solution of N-nitrosomethylurea prepared as described above. Sixteen control animals were given a corresponding volume of buffer solution alone. Blood samples were taken 1, 3 and 6 hours after the injection and also after 1, 2, 3, 4 and 7 days. Tests for glucosuria were made with glucose-oxidase paper strips (Clinistix®, Ames, Co., London, England). In animals dying spontaneously, as well as in 17 animals killed after 0.5, 1, 3, 5, 6 and 24 hours, specimens were taken from the pancreas, liver and kidneys for light microscopic analyses. Seven Chinese hamsters were given 500 mg/kg body weight of nicotinamide intraperitoneally 10 minutes before the injection of N-nitrosomethylurea. The nicotinamide was dissolved in distilled water at a concentration of

10% (w/v) immediately before use. Five controls were given 500 mg/kg body weight of nicotinamide alone. Blood samples were taken from these animals after 2 days for blood glucose analysis.

The specimens taken for light microscopic examination were fixed in Bouin's solution or in 10% (v/v) formalin, dehydrated and embedded in paraffin or butoxy ethanol-glycol methacrylate (AB LAMBDA, Stockholm, Sweden). The paraffin embedded material was sectioned in 4 µ thick sections and the methacrylate embedded material in 1 µ thick sections (Sorvall IB-4 microtome). The following stains were used: Hematoxylin-eosin, van Gieson's stain, aldehyde fuchsin, and silver impregnations according to Grimelius[12] and Hellerström and Hellman[13].

RESULTS

L.D. 50 and Mortality

A linear relationship was found between the log dose of intraperitoneally injected N-nitrosomethylurea and the mortality when plotted on a log-probability scale (Fig. 1). The approximate L.D. 50 of intraperitoneally injected N-nitrosomethylurea at an observation time of 48 hours was about 125 mg/kg body weight. Fig. 2 shows the cumulated mortality after injection of 50 mg/kg body weight of N-nitrosomethylurea. The curve was sigmoid and quite steep during the 3-7 day period following the injection. Nine days after the injection 90% of the animals were dead.

Blood Glucose Concentration

After the intraperitoneal injection of 50 mg/kg body weight of N-nitrosomethylurea in non-fasting Chinese hamsters, a triphasic

blood glucose curve was obtained (Fig. 3). There was an initial hyperglycemic peak with a maximum after 3 hours (162 ± 11 mg/100 ml M ± SEM, $p < 0.01$), followed by a decrease with a minimum at 6 hours (85 ± 9 mg/100 ml). After 24 hours moderately elevated blood glucose values were again recorded (141 ± 17 mg/100 ml, $p < 0.05$), with a further slight increase during the following day (159 ± 11 mg/100 ml, $p < 0.01$). No obvious decrease in the blood glucose concentration was noted up to the end of the experimental period. The control animals were normo-glycemic at all observation times. The experimental animals with hyperglycemia often showed glucosuria. No glucose was observed in the urine of the control animals.

Nicotinamide

Pretreatment with 500 mg of nicotinamide/kg body weight inhibited the blood glucose elevation evoked by N-nitrosomethylurea, as seen in Table I. The difference in blood glucose concentration between the N-nitrosomethylurea and nicotinamide + N-nitrosomethylurea urea injected animals was significant after 2 days ($p < 0.01$). No significant difference in blood glucose concentration was recorded between animals injected with buffer alone, nicotinamide and nicotinamide + N-nitrosomethylurea ($p > 0.1$).

Pancreatic Islet Light Microscopic Morphology

Thirty minutes after injection of 50 mg/kg body weight of N-nitrosomethylurea no changes were observed in the pancreatic islets. At one hour there was slight vacuolization in a few islet cells. After three hours nuclear pyknosis and cytoplasmic vacuolization were seen in β-cells (Fig. 5), as well as in α_2- and α_1-cells as revealed by silver impregnations according to Grimelius[12] and Hellerström and Hellman[13]. No obvious degranulation occurred. During the following hours there was pronounced derangement, with karyorrhexis and

fragmentation of the islet endocrine cellular structures. The changes in the islet tissue varied to some extent in different animals, but after 24 hours this tissue contained only amorphous material and a few endothelial cells but no endocrine cells (Fig. 6). No obvious light microscopic changes occurred in islet tissue of buffer treated controls (Fig. 4). Neither were there any changes in the kidneys nor in the exocrine panreatic tissue of the experimental animals. In the liver fatty metamorphosis was found in some cases.

DISCUSSION

In the present study injection of 50 mg of N-nitrosomethylurea/kg body weight in Chinese hamsters resulted in initial hyperglycemia at 3 hours, a blood glucose decrease at 6 hours and moderately elevated values during the following days. Streptozotocin injected Chinese hamsters also show a triphasic blood glucose curve within one day[5]. The initial blood glucose elevation after N-nitrosomethylurea might be explained by an inhibition of insulin release, an effect which both streptozotocin[14] and N-nitrosomethylurea exhibit in isolated islets[8]. The hypoglycemia after 6 hours may, on the other hand, be caused by insulin liberation from β-cells, which at that time have reached the stage of disintegration. The marked transient hyperglycemia observed in streptozotocin-diabetic Chinese hamsters after one day[10] was not found in the N-nitrosomethylurea injected animals. No simple explanation for this difference cannot be offered at present.

Since the L.D. 50 for N-nitrosomethylurea (mol. weight 103) at an observation time of 48 hours was found to be 125 mg/kg body weight, it seems to be considerably more toxic in Chinese hamsters than streptozotocin (mol. weight 265), which has an L.D. 50 value of 400 mg/kg

body weight[5].

Similarities exist between streptozotocin and N-nitrosomethylurea in that both substances give a decrease in the NAD concentration in pancreatic islet cells. This effect can be prevented by pretreatment with nicotinamide[8,9]. In Chinese hamsters[10], like other laboratory rodents[7,15], the induction of streptozotocin diabetes can be inhibited by large doses of nicotinamide, indicating that depletion of cellular NAD may be causally related to the β-cell deterioration in this animal. This also seems to be valid for N-nitrosomethylurea diabetic Chinese hamsters, in that no blood glucose elevation occurred in animals injected with 500 mg/kg body weight of nicotinamide 10 minutes before the administration of N-nitrosomethylurea.

The light microscopic changes in the pancreatic islets observed after injection of diabetogenic doses of streptozotocin and N--nitrosomethylurea seem to be similar, since they occur at about the same time after injection and are seen in all types of islet cells. The results indicate that destruction of the pancreatic islet tissue, and mainly the β-cells, probably plays a major role in the development of diabetes in N-nitrosomethylurea injected Chinese hamsters. There seem, however, to be species differences in regard to the β-cytotoxic action of N-nitrosomethylurea, since β-cell destruction in mice could only be induced with very high doses of N-nitrosomethylurea (230 mg/kg body weight[8]) with a high mortality, rendering it difficult to study any development of permanent hyperglycemia in these animals.

Species differences among laboratory rodents have also been found for the effects of other β-cytotoxic substances. Guinea pigs, for example, are reported to be resistant to the diabetogenic action of alloxan[16] or to demand high doses of streptozotocin for induction

of β-cell necrosis[17-18].

Thus the glucose moiety of the streptozotocin molecule obviously is not necessary for induction of diabetes in Chinese hamsters, but streptozotocin seems to be a more useful diabetogenic substance for experimental studies, since the general toxic effects of N--nitrosomethylurea rule out long observation periods and, for instance, studies on possible islet cell regeneration.

Beside the strong diabetogenic effects of N-nitrosomethylurea observed with the present doses it cannot be excluded that prolonged exposure to smaller doses of this compound could be injurious to the β-cells. This drug belongs chemically to the nitrosamines, some of which occur in low concentration in a wide variety of food products[19] or can be synthesized in the mammalian stomach from various amine precursors and nitrite[20]. Many of the N-nitroso compounds have carcinogenic properties and the environmental nitrosamines have been considered as potentially carcinogenic for man[21]. With regard to the present result, attention must now also be directed towards a possible diabetogenic effect of nitrosamines in humans.

ACKNOWLEDGEMENT

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TABLE I. The effect of nicotinamide on N-nitrosomethylurea induced hyperglycemia in Chinese hamsters. The blood glucose concentrations were determined 48 hours after injection.

Treatment	Blood glucose mg/100 ml M ± SEM
N-nitrosomethylurea 50 mg/kg	159 ± 11[x] n = 14
Nicotinamide 500 mg/kg + N-nitrosomethylurea 50 mg/kg	109 ± 10 n = 7
Nicotinamide 500 mg/kg	98 ± 6 n = 5
Buffer	96 ± 3 n = 16

[x] $p < 0.01$ in comparison with animals treated with nicotinamide + N-nitrosomethylurea.

Fig. 1. Dose - mortality curve after intraperitoneally administered N--nitrosomethylurea plotted on a log-probability scale.

Fig. 2. Cumulated mortality in per cent in 19 Chinese hamsters injected with 50 mg N-nitrosomethylurea/kg body weight.

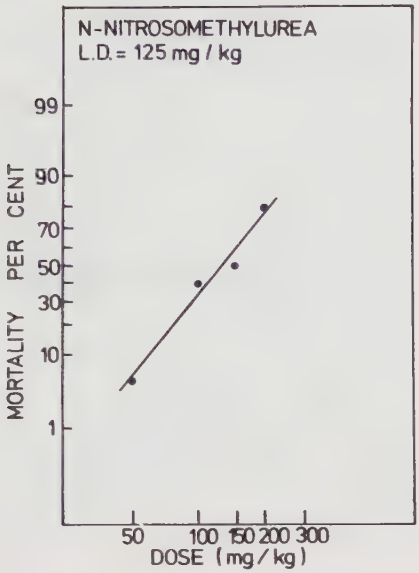
N-NITROSOMETHYLUREA
L.D.= 125 mg / kg
MORTALITY PER CENT
99
90
70
50
30
10
1
50
100
150
200
300
DOSE (mg/kg)

Fig. 1

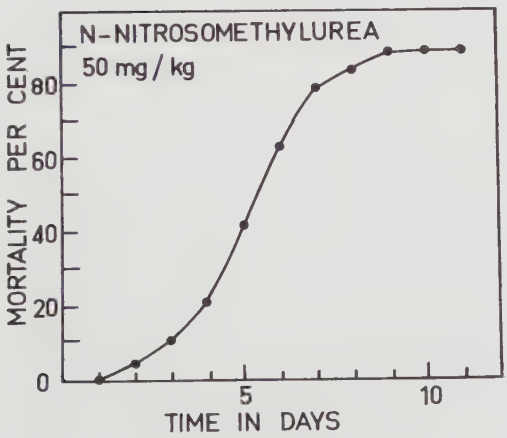
N-NITROSOMETHYLUREA
50 mg / kg
MORTALITY PER CENT
80
60
40
20
0
5
10
TIME IN DAYS

Fig. 2

Fig. 3. Blood glucose variations (M ± SEM) in 14 N-nitrosomethylurea (50 mg/kg) injected Chinese hamsters and 16 controls.

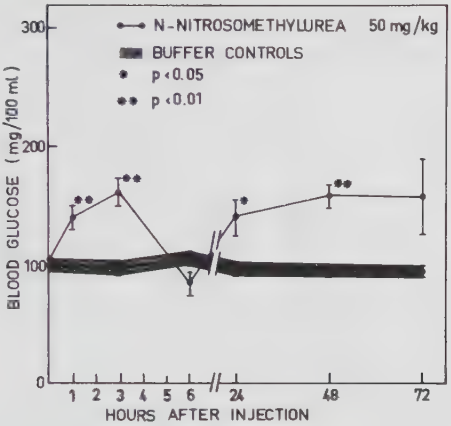
N-NITROSOMETHYLUREA 50 mg/kg
BUFFER CONTROLS
p<0.05
p<0.01
BLOOD GLUCOSE (mg/100 ml)
300
200
100
0
1 2 3 4 5 6 24 48 72
HOURS AFTER INJECTION

Fig. 3

Fig. 4. Pancreatic islet in a control animal one day after injection of buffer solution. Hematoxylin-eosin x 640.

Fig. 5. Pancreatic islet 3 hours after injection of 50 mg N-nitrosomethylurea/ /kg body weight showing nuclear pyknosis and cytoplasmic vacuolization. Hematoxylin-eosin x 640.

Fig. 6. Pancreatic islet one day after injection of 50 mg N-nitrosomethylurea/ /kg body weight, showing total destruciton of endocrine cellular elements in the islet tissue. Hematoxylin-eosin x 640.

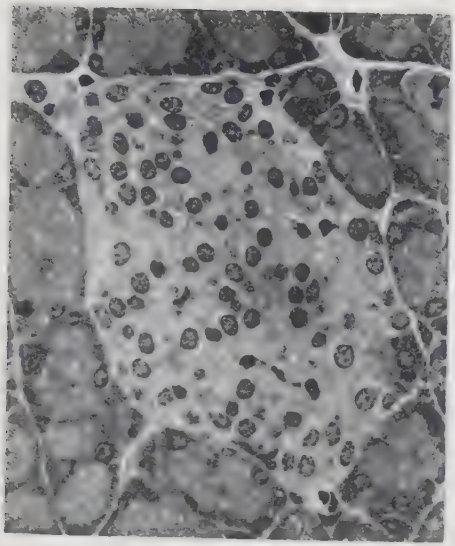

Fig. 4

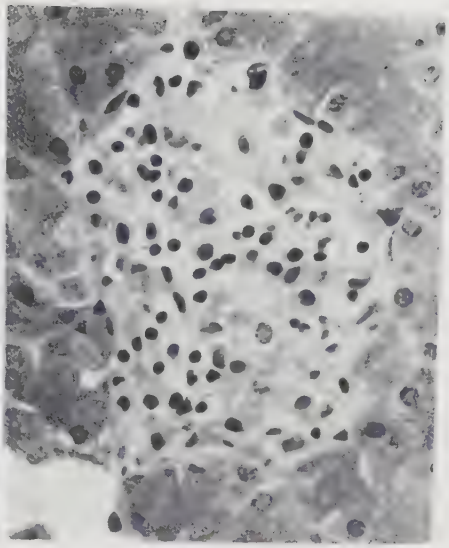

Fig. 5

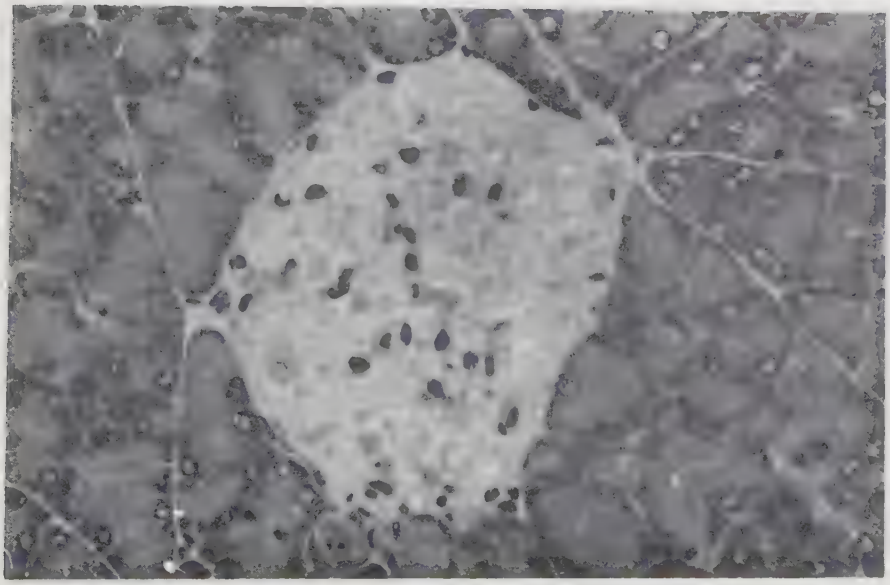

Fig. 6

An Ultrastructural Study of Pancreatic Islet Cell Destruction by N-nitrosomethylurea.

by

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Running title: Pancreatic Islet Cell Destruction by N-nitrosomethylurea.

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SUMMARY

An ultrastructural study of the early effects of intraperitoneal injection of 50 mg/kg body weight of N-nitrosomethylurea on the pancreatic islet tissue was performed in Chinese hamsters. Thirty minutes after injection there was slight dilatation of the endoplasmatic reticulum and mitochondrial swelling in a few peripheral α_2-cells near to capillaries and also in some β-cells. After 1 hour these changes were more pronounced. Distinct nuclear pyknosis was seen after 3 hours in endocrine islet cells of all types, together with β-cell granular irregularities, disruption of granular cores and autophagy of α_2-cell granules. During the following hours marked derangement of intracellular structures, ending in obvious cellular destruction, was observed. The results indicate that N-nitrosomethylurea has a direct cytotoxic effect on the β-, α_2- and α_1-cells of the islet tissue. The similarity between the ultrastructure of the pancreatic islets after injection of N-nitrosomethylurea and streptozotocin is discussed.

Key words: Chinese Hamster - N-nitrosomethylurea - Pancreatic Islet Tissue - Ultrastructure

INTRODUCTION

In Chinese hamsters not only the well known diabetogenic substance streptozotocin[1] but also the N-nitrosomethylurea moiety of the streptozotocin molecule has been shown to produce overt diabetes[2], but the latter substance seems to be a less useful agent in experimental diabetic research when longer observations periods are desired, as its toxic effects cause death of most diabetic animals within one week[2]. However, it would seem of value to compare the functional and morphological evolution of the experimental diabetic states caused by injection of these two substances in order to elucidate their mechanisms of action. Light microscopic studies and determination of early blood glucose changes after injection of streptozotocin and of N-nitrosomethylurea in Chinese hamsters have shown similar pictures. Both substances seem to affect the β-, α_2- and α_1-cells of the islet tissue. Blood glucose elevations and islet cell destruction can be inhibited by pretreatment with nicotinamide[2,3].

The purpose of the present investigation was to study the ultrastructural morphology in the pancreatic islet tissue after injection of N-nitrosomethylurea in Chinese hamsters.

MATERIAL AND METHODS

Sixteen Chinese hamsters of both sexes, five months old and weighing 25-38 gm, were used. They were selected at random from families in which no overt diabetes had been observed for more than six generations. Before the start of the experiment it was

checked that the animals were free from glucosuria. They were fed a conventional diet for laboratory rodents and were given tap water ad libitum.

Fourteen animals were injected intraperitoneally with N-nitroso-methylurea (Pfaltz and Bauer, Flushing, New York, USA) in a dose of 50 mg/kg body weight. The substance was dissolved immediately before use in a citrate phosphate buffer, pH 4.0, in a concentration of 2%. Two animals were given a corresponding volume of plain buffer solution intraperitoneally.

In ether anæsthetized animals an incision about 2 cm long was made in the abdominal wall and biopsies were taken from the pancreas for electron microscopic examination, after which the abdominal wall was closed in the animals which were not killed. One, two or three biopsies were taken in this way, totalling 22 altogether. Immediately after the last biopsy the animals were killed and specimens from the rest of the pancreas were taken for light microscopic examination. They were fixed in Bouin's solution, embedded in paraffin and sectioned in 4 µ thick sections which were stained with van Gieson's stain.

The biopsies for ultrastructural analyses were taken 15 minutes to 6 hours after the injection of N-nitrosomethylurea. They were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 4 hours. After rinsing in 0.1 M sucrose, the specimens were postfixed in 1% osmium tetroxide for 90 minutes. All steps in the fixing process took place at $+4^{\circ}C$. The specimens were dehydrated and embedded in Epon 812[4]. After localization of islets in approximately 1 µ thick toluidine blue stained sections, ultrathin sections were prepared with an LKB Ultrotome. The sections were mounted

on Formvar-coated copper grids, contrasted with uranyl acetate[5] and lead citrate[6] and examined in a Zeiss electron microscope EM 9 at 60 kV.

RESULTS

Light Microscopic Morphology

Specimens from the pancreas taken at sacrifice from the animals injected with N-nitrosomethylurea showed islet cell degeneration and necrosis similar to those described previously[2]. In the buffer controls no changes were noted in the islet tissue.

Electron Microscopic Morphology

Fifteen minutes after injection of N-nitrosomethylurea all islet cells appeared normal. The first alterations were seen after 30 minutes, consisting in slight mitochondrial swelling and dilatation of the endoplasmatic reticulum in a few α_2-cells (Fig. 1) near to capillaries as well as in some β-cells (Fig. 2).

At 1 hour the mitochondrial swelling and intracytoplasmic vacuolization were more pronounced in the α_2-cells and β-cells. β-cells containing intracytoplasmic dense bodies (Fig. 3) and α_2-cells with secretory granular cores incorporated in lysosomes (granulophagy) (Fig. 4) were also seen at this time point, as well as granular material in the endoplasmatic reticulum of some α_2-cells.

Three hours after injection of N-nitrosomethylurea distinct condensation of nuclear chromatin was observed in β-, α_2- and α_1-cells (Figs. 5 and 6). At the same time and during the following hours the β-cell granule limiting membranes sometimes ruptured and the

secretory granular cores showed marked irregularities and even disappearance, leaving an electron lucent space (Fig. 7). No degranulation occurred. In the α_2-cells dilatation and even rupture of the granule limiting membrane occurred (Fig. 8).

At the end of the observation period the degenerative changes were more pronounced, with nuclear karyorrhexis and marked disintegration of intracytoplasmic structures, resulting in cellular destruction. Because of the pronounced changes it was often difficult at that time to identify the different types of cells in the islets.

During the later part of the experimental period macrophages with engulfed granules from disintegrated endocrine islet cells were seen. This granulophagy differed from that observed earlier in α_2-cells in that the granule limiting membranes were sometimes preserved after incorporation into the lysosomes (Fig. 9).

No obvious alterations were found in exocrine pancreatic tissue of N-nitrosomethylurea treated Chinese hamsters. In endocrine pancreatic tissue of buffer injected animals no alterations including dense bodies in the cytoplasm of β-cells were found.

The repeated biopsies of the pancreas in some animals did not seem to affect the islet cell morphology, since the same picture was observed in pancreases from which only one biopsy was taken.

DISCUSSION

No other reports appear to have been made on the ultrastructural morphology of the pancreatic islet tissue after injection of

N-nitrosomethylurea. In streptozotocin injected rats Arison et al.[7] found β-cell degranulation without obvious necrosis, and Lazarus and Shapiro[8] observed dilated endoplasmatic reticulum and swollen mitochondria in rabbit β-cells and α_2-cells after 3 hours, followed later by necrosis of these cells. In guinea pigs, on the ultrastructural level streptozotocin causes a selective destruction of the islet β-cells, leaving the α_2- and α_1-cells completely intact[9]. The first ultrastructural changes in the pancreatic islets of Chinese hamsters after injection of streptozotocin occur after about 30 minutes and consist of aggregates of secretory granules in some β-cells, which have also been described in Chinese hamsters with spontaneous hereditary diabetes[10]. At 1 1/2 hours cytoplasmic vacuolization of varying degrees and mitochondrial swelling are seen in the streptozotocin injected animals. Pyknosis occurs in β-cells after 3 hours in these animals, together with disruption of granule limiting membranes and irregularities or disappearance of granular cores. Degenerative changes also take place in α_2-cells and lysosomes with incorporated granules are seen in both β- and α_2-cells[1]. The degenerative changes in the streptozotocin injected Chinese hamster greatly resemble those observed after injection of N-nitrosomethylurea in the same animal except for autophagy of granules in the β-cells in the former. The time courses of the destruction of islet endocrine cells by streptozotocin and N-nitrosomethylurea seem to be about the same.

Similarities in the ultrastructural morphological changes in islet tissue caused by streptozotocin and N-nitrosomethylurea may indicate that their mode of action in this tissue is identical. However, the ultrastructural alterations are non-specific and are also found in pancreatic islet β-cells in alloxan treated Chinese hamsters,

although there they appear after only 5 minutes. The difference between alloxan, on the one hand, and streptozotocin and N-nitrosomethylurea, on the other, is obvious, however, since alloxan only causes slight changes in α_2- and α_1-cells, which have been explained as secondary to the extensive β-cell damage[11]. The present study indicates that N-nitrosomethylurea has a direct cytotoxic effect on the islet tissue β-, α_2- and α_1-cells in Chinese hamsters, as is also probable in the case of streptozotocin.

It may be of interest to note that the specificity of streptozotocin for β-cells varies in different animal species[1,8,9]. The fact that both streptozotocin and N-nitrosomethylurea destroy all types of endocrine cells of the islet tissue in Chinese hamsters may favour the hypothesis of Pearse, Polak and Heath[12] that all these cells arise from common precursor cells of neuroectodermal origin. However, whether or not the cytotoxic action of streptozotocin and N-nitrosomethylurea on the α-cells is a result of lowering of cellular nicotinamide adenine dinucleotide levels, as is probable in the β-cells[13], is not known.

Autogranulophagy, which has been interpreted as lysosomal digestion of unneeded secretory granules[14], has been described in pancreatic islet α_2-cells in spontaneously diabetic animals[15,16], in streptozotocin diabetic rats[15] and Chinese hamsters[1], and also, but rarely, in normal hamsters[16]. Further evidence supporting the assumption that autogranulophagy represents "auto digestion" of unneeded secretory products is given by Creutzfeldt et al.[17], who found autophagy of β granules after inhibition of insulin release by diazoxide in rats. In the present study granules incorporated into lysosomes were seen in α_2-cells after injection of N-nitrosomethylurea. Golden et al.[18] and Gunnarsson, Berne, and Hellerström[13]

have shown that streptozotocin exerts an inhibitory effect on insulin release in vitro. It is possible that N-nitrosomethylurea and streptozotocin have a similar effect on the glucagon release in α_2-cells.

ACKNOWLEDGEMENT

This work was supported by grants from the Swedish Medical Research Council (Project Nos. 102 and 718) and the Swedish Diabetes Association.

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Fig. 1. An α_2-cell 30 minutes after injection of N-nitrosomethylurea, with mitochondrial swelling and dilatation of the endoplasmatic reticulum. X 17.7[illegible].

Fig. 2. β-cells 30 minutes after injection of N-nitrosomethylurea, with swollen mitochondria and intracytoplasmatic vacuolization. X 17.700.

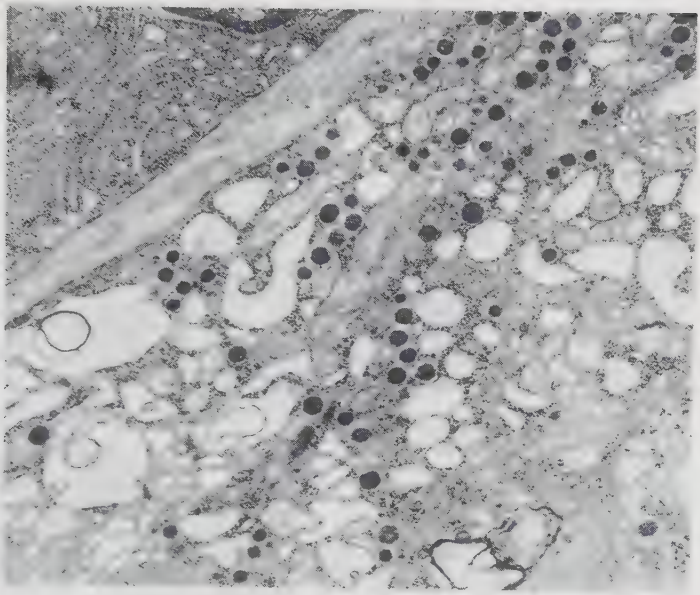

Fig. 1.

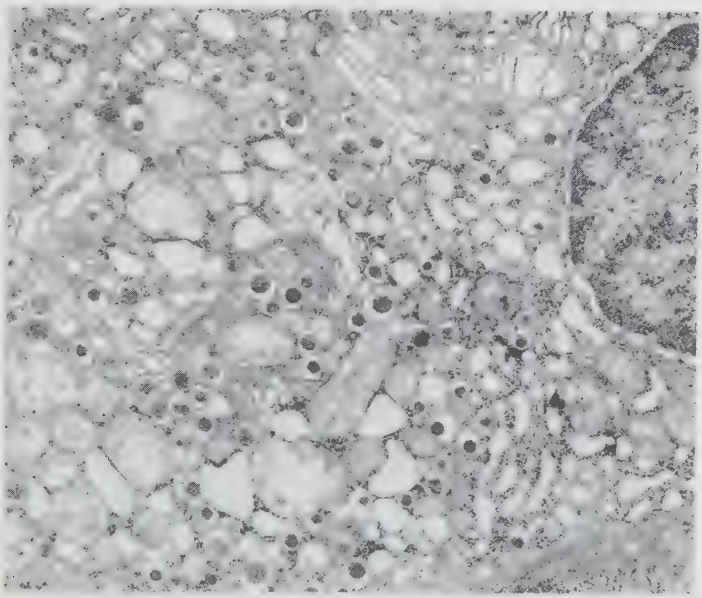

Fig. 2.

Fig. 3. Dense bodies at least partly surrounded by limiting membranes in a β-cell cytoplasm 1 hour after injection of N-nitrosomethylurea. X 17.700.

Fig. 4. Peripherally located α_2-cell one hour after injection of N-nitrosomethylurea with typical secretory granules, some of which have lost their limiting membranes and are incorporated in lysosomes. X 17.700.

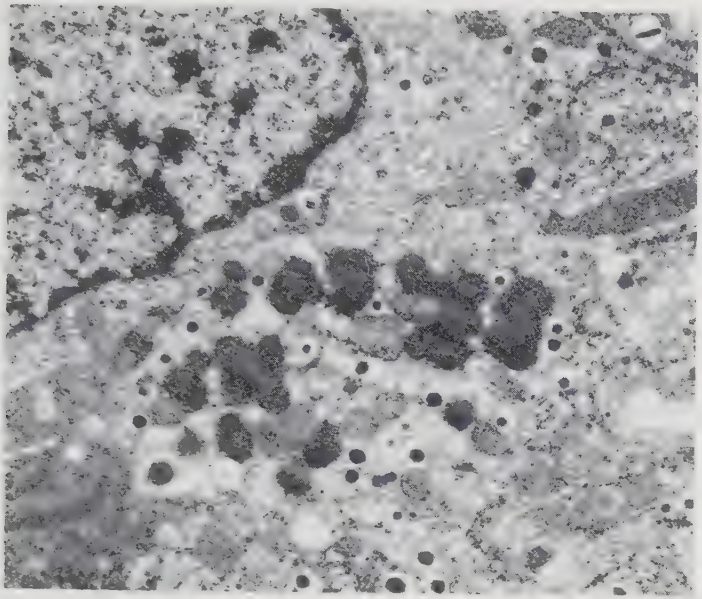

Fig. 3.

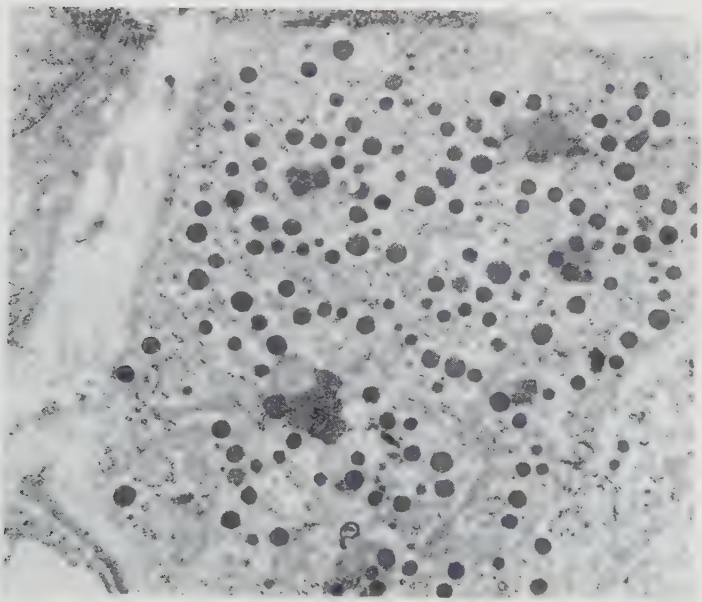

Fig. 4.

Fig. 5. Part of pancreatic islet 3 hours after injection of N-nitrosomethylurea, showing nuclear pyknosis in a β-cell (right), an α_2-cell (middle) and an α_1-cell (left). Intracytoplasmic vacuolization of the β- and α_2-cells is also seen. X 9.100.

Fig. 6. Part of an α_1-cell 3 hours after injection of N-nitrosomethylurea, with typical secretory granules, slight mitochondrial swelling and condensation of nuclear chromatin. X 17.700.

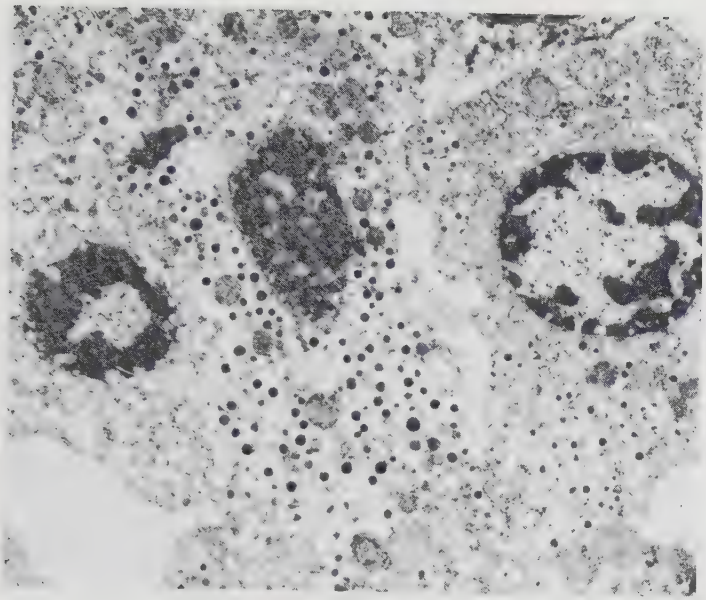

Fig. 5.

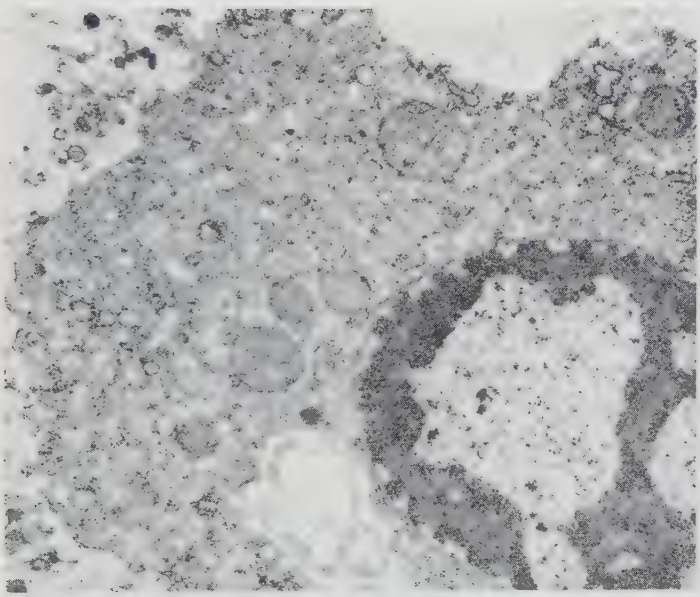

Fig. 6.

Fig. 7. Pancreatic islet β-cell 5 hours after injection of N-nitrosomethylurea. Nuclear pyknosis, irregularities or disappearance of granular cores, disruption of granular membranes and mitochondrial swelling are seen. X 17.700.

Fig. 8. Peripherally located α_2-cell 5 1/2 hours after injection of N-nitrosomethylurea. Nuclear pyknosis and secretory granules with dilated or disrupted limiting membranes are seen. X 17.700.

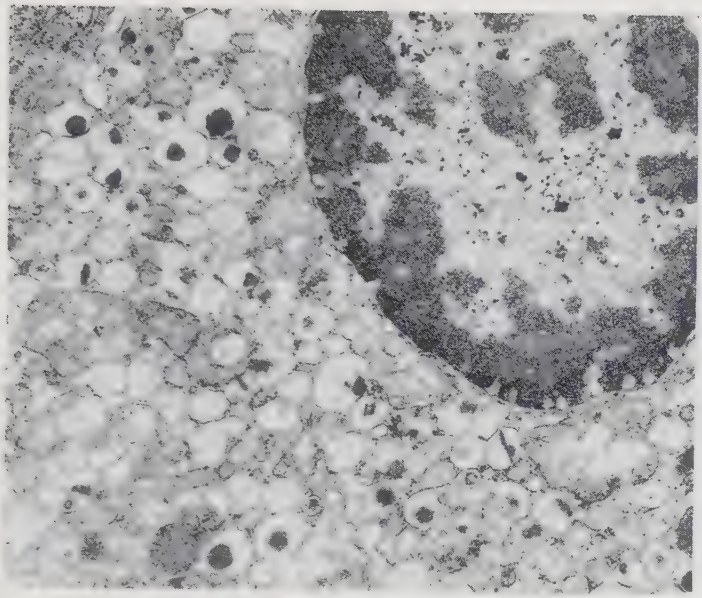

Fig. 7.

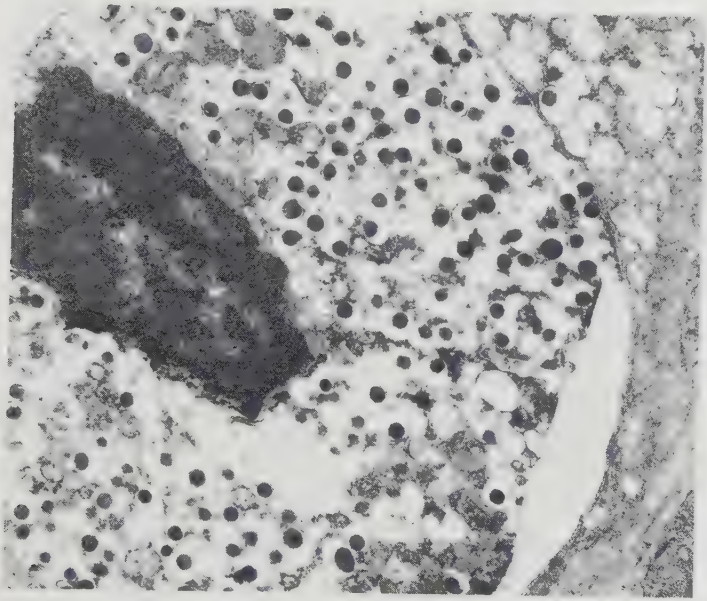

Fig. 8.

Fig. 9. Macrophage in pancreatic islet 5 hours after injection of N-nitroso-methylurea with phagocytosis of endocrine granular material. X 17.700.

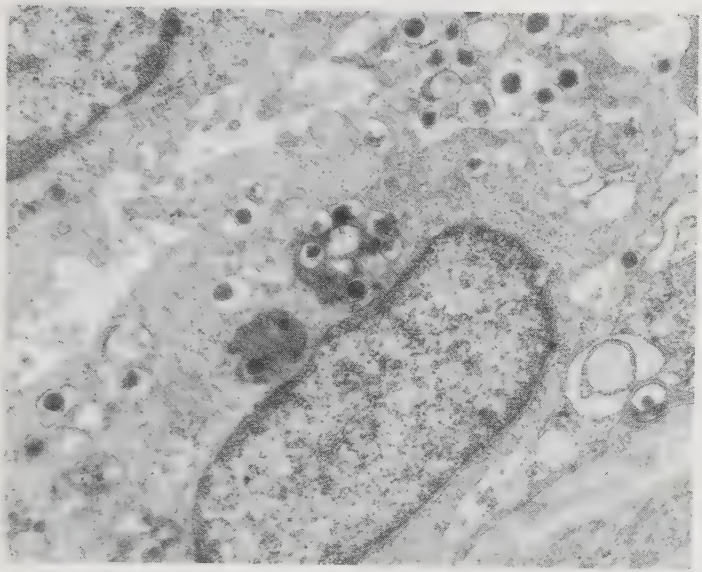

Fig. 9.

On the ability of N-nitrosomethylurea to be diabetogenic by accumulating in the pancreatic islets.

by

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INTRODUCTION

N-nitrosomethylurea belongs to the N-nitroso compounds, a group of substances which have powerful biological properties including carcinogenicity, mutagenicity and teratogenicity[1]. There are several reports that small amounts of N-nitroso compounds may be present in the human environment or may be formed endogenously under conditions existing in the human stomach[2,3].

N-nitrosomethylurea is also an aglucone of the well known diabetogenic substance streptozotocin[4]. It has been suggested that the addition of glucose to N-nitrosomethylurea results in a molecule that can enter and destroy the pancreatic β-cells[5]. Recently, however, it has been shown that islet tissue β-cells can be damaged by N-nitrosomethylurea alone, although species differences seem to exist. Thus, in Chinese hamsters doses of about 50 mg/kg body weight of N-nitrosomethylurea result in islet cell damage and hyperglycaemia[6]. In mice, on the other hand, β-cell destruction can only be obtained with high doses of N-nitrosomethylurea (230 mg/kg) which cause severe general toxic effects and death, making it impossible to study the possible development of hyperglycaemia[7]. The reason for the species differences remains unclear.

It has been suggested that the β-cell deterioration caused by streptozotocin and N-nitrosomethylurea may be aetiologically related to depletion of cellular nicotine adenine dinucleotide (NAD), since both are capable of lowering the β-cell NAD level[7,8]. Pre-treatment with nicotinamide inhibits the β-cytotoxic effect of both streptozotocin[9-10] and N-nitrosomethylurea,[6] probably by protecting against a decrease in the cellular NAD.

In the present study the uptake of N-nitrosomethylurea in the pancreatic islets of Chinese hamsters and two strains of mice was investigated. The effect of pre-treatment with nicotinamide on the uptake of N-nitrosomethylurea in islet tissue was also studied. Blood glucose levels after administration of N-nitrosomethylurea were used as an indicator of the diabetogenic effect of this compound. This investigation was performed with the aim of obtaining further information on the mode of action of N-nitrosomethylurea in the pancreatic islets.

Key words: Chinese hamster - Mice - Pancreatic islets - ^{3}H-N--nitrosomethylurea - Nicotinamide - Autoradiography

MATERIAL AND METHODS

Blood glucose determination

Thirty-five non-diabetic Chinese hamsters and 20 mice of the C57-Bl strain weighing about 25 gm each were used. They were injected intraperitoneally with 50, 100 and 150 mg/kg body weight of N-nitrosomethylurea (Pfaltz and Bauer Flushing, New York, USA) or a corresponding volume of plain buffer solution. The N-nitrosomethylurea was dissolved in a citrate-phosphate buffer, pH 4.0, at a concentration of 2% (w/v) immediately before use.

Blood samples were obtained after 2 days by the orbital bleeding technique[11]. The animals were anæsthetized with ether prior to the puncture, which was performed with heparinized microhematocrit tubes (Drummond Scientific Company, USA) in the left orbital venous plexus. The blood glucose concentration was determined by a glucose-oxidase method with the aid of a commercially available kit (AB Kabi, Stockholm, Sweden).

Autoradiography

Nine adult non-diabetic Chinese hamsters, 5 mice of the C57-Bl strain, and 2 mice of the recessively inherited obese-hyperglycaemic strain (one obese and one from a lean litter) were used. The Chinese hamsters and the lean mice weighed about 25 gm each, and the obese-hyperglycaemic mouse 50 gm. All the animals were fed a conventional diet for laboratory rodents and were given tap water ad libitum.

The animals were divided into three groups.

In the first group 5 Chinese hamsters and 7 mice were each injected intravenously with 0.5 mCi (corresponding to 40 mg/kg body weight for Chinese hamsters and lean mice and 20 mg/kg for the obese-hyperglycaemic mouse) of ^{3}H-N-nitrosomethylurea (labelled at the methyl group, spec. act. 48 mCi/mM, New England Nuclear, Boston, USA), dissolved in 0.2 ml of a citrate phosphate buffer[12], pH 4.0[13]. The mice were injected in a tail vein while the Chinese hamsters were injected in the jugular vein after being slightly anæsthetized with ether. One Chinese hamster and one C57-Bl mouse were then killed 5 minutes, 30 minutes, 1 hour, 4 hours, and 24 hours after the injection by being anæsthetized with ether and frozen in hexane cooled with solid CO_2 to $-78^{\circ}C$. At the one-hour survival time the obese and the non-obese mouse of the recessively obese-hyperglycaemic strain were also killed along with the other animals. After freezing, the animals were embedded in a mixture of carboxymethyl cellulose and water. Autoradiography was then performed as described by Ullberg[14-15]. Thus sagittal sections (20 µ thick) of the whole animals were cut on tape (Minnesota Mining and Manufacturing Co., USA; tape No. 800) at $-15^{\circ}C$ in a microtome. The sections were then freeze-dried at $-15^{\circ}C$ and autoradiograms were obtained by attaching the sections to photographic plates (G5, Ilford). After an exposure time

of about 1 month the photographic plates were developed in Kodak D19 and fixed in Gevaert G305 A. The sections were stained with hematoxylin-eosin for the identification of structures in the autoradiograms.

The second group of animals consisted of three Chinese hamsters. Each animal was given an intraperitoneal injection of 500 mg/kg nicotinamide (AB Kebo, Stockholm, Sweden). Ten minutes later they were injected intravenously with 0.5 mCi ^{3}H-N-nitrosomethylurea. They were then killed after 30 minutes, 1 hour and 4 hours and were examined autoradiographically as described for the first group.

In the third group one Chinese hamster was injected intravenously with 0.5 mCi ^{3}H-N-nitrosomethylurea. After one hour the animal was killed by cervical dislocation. Pieces of the pancreas were quickly removed, frozen in isopentane, cooled with liquid nitrogen, freeze--dried at -40°C in a Speedivac-Pearse tissue dryer for 5 days and then embedded in paraffin. 5-7 µ thick sections were then cut on tape (MMM, No. 688) and microautoradiography was performed according to Hammarström et al.[16]. This includes attachment of the tape sections to photographic plates (K2 and G5, Ilford), exposure at -10°C (the exposure time being about 2 weeks) and removal of the tape from the sections in xylol. The photographic plates were developed for 3 minutes in Kodak D19, fixed and thereafter stained with hematoxylin-eosin and in some cases also with aldehyde fuchsin according to Jennings[17] - as described by Tjälve[18] - for identification of the β-cells in the pancreatic islets.

RESULTS

Blood glucose

The results of the blood glucose analyses are given in Table I. As seen, there is a significant difference in the blood glucose concentration ($p < 0.001$) between the Chinese hamsters injected with N-nitrosomethylurea (50 and 150 mg/kg) and the control animals injected with the buffer solution alone. In the mice no blood glucose elevation was noted after any of the injected doses of N-nitrosomethylurea. Thus, in mice injected with 50, 100 and 150 mg/kg body weight of N-nitrosomethylurea the blood glucose concentrations did not differ from those of the animals injected with buffer solution alone ($p > 0.1$).

Autoradiography

Chinese hamsters

In the whole body autoradiograms radioactivity was seen to be accumulated in the pancreatic islets 30 minutes and 1 hour after the injection of ^{3}H-N-nitrosomethylurea (Figs. 1-2). The uptake was most pronounced after 1 hour, at which survival time the radioactivity in the pancreatic islets, together with the liver, was highest in the body. At later survival times the radioactivity in the pancreatic islets did not exceed that in the exocrine pancreas. Microautographic examination of the pancreas one hour after injection of labelled N-nitrosomethylurea revealed a strong accumulation of silver grains over the islet cells. Accumulation of the isotope was seen in both the β- and the α-cells. The silver grains were found mainly over the nuclei of the cells, while in the cytoplasm they were considerably less numerous (Fig. 3). There were fewer silver grains over the exocrine pancreas than over the islets. The

labelling in the exocrine pancreas, as in the islets, was most marked over the nuclei. Pre-treatment with 500 mg nicotinamide/kg body weight did not cause any appreciable alterations in the concentration of labelling in the pancreatic islets (Fig. 4).

Mice

Throughout the entire experimental period the accumulation of radioactivity in the islet tissue of mice was low, irrespective of strain. Thus, both in the C57-Bl mice and in the obese and lean mice of the obese-hyperglycaemic strain the uptake of isotope in the islets was lower than in the exocrine pancreas or in the blood (Fig. 5).

DISCUSSION

This investigation has shown that there are species differences in the uptake of N-nitrosomethylurea in the pancreatic islets. An accumulation was found in the Chinese hamsters but not in the mice. Blood glucose elevations after the injection of N-nitrosomethylurea were found in the Chinese hamsters, but not in the mice. It thus seems that the ability of N-nitrosomethylurea to be specifically accumulated by the islet tissue in Chinese hamsters but not in mice reflects its ability to produce hyperglycemia and diabetes in the former animals. However, in mice very high doses of N-nitrosomethylurea (230 mg/kg) are capable of causing β-cell destruction which then is combined with severe general toxic effects, rendering it impossible to study any development of hyperglycemia[7]. In the Chinese hamsters a dose of 50 mg/kg body weight has been shown to destroy all types of islet cells[6]. The reason for these differences in accumulation is obscure. They may reflect some difference between mice

and Chinese hamsters in regard to the transport systems or the intracellular metabolism of the islet tissue. It has been claimed that in streptozotocin the glucose moiety is important for the toxic effect on the pancreatic islets by facilitating the transport of the molecule to the islets[5]. The results of this investigation underline the importance of a high accumulation in the islets for a β-cytotoxic effect.

Pre-treatment with nicotinamide protects the β-cells against the noxious action of N-nitrosomethylurea[6]. In the light of this knowledge it seemed of interest to determine whether administration of nicotinamide prior to an injection of labelled N-nitrosomethylurea could prevent the accumulation of the latter in the pancreatic islets of Chinese hamsters. However, no obvious alteration of this accumulation was noted. Thus nicotinamide does not appear to exert its protective action against N-nitrosomethylurea by altering the ability of the latter to reach its target in the cells.

Within the islets the highest radioactivity was found over the nuclei of the cells. Administration of N-nitrosomethylurea causes nuclear damage in Chinese hamster islet cells at a relatively early stage[19], indicating that it interferes with some nuclear component. N-nitrosomethylurea is an alkylating carcinogen, causing preferentially 7-alkylation of guanine in DNA and RNA[20]. On the other hand, the action of many alkylating agents takes place in some part of the NAD biosynthesis[21]. NAD biosynthesis is confined to the nuclei of the cells[22]. The protective action of nicotinamide against the islet cell toxicity caused by N-nitrosomethylurea and streptozotocin[6,9-10] suggests that destruction of the islet cells by these two substances occurs through a decrease in the availability of nicotinamide for synthesis of NAD. An effect on both nuclear DNA-RNA and NAD biosynthesis seems possible as an explanation for the cell damage, but

the mechanism involved must be investigated further.

The finding that labelled N-nitrosomethylurea accumulates in both the β- and α-cells of the Chinese hamster is in accordance with the observations on the pancreatic islet ultrastructure in this animal, i.e. that changes occur in both the β- and α-cells after administration of this substance[6,19]. The highest accumulation of labelled N-nitrosomethylurea was observed after one hour, after which the radioactivity decreased. This is probably explained by the islet cell destruction, as it has been shown that 50 mg/kg of this substance - approximately the same dose as was used in this investigation - causes a total destruction of the β-, α_2- and α_1--cells in the Chinese hamster after about 6 hours[6,19].

Since the recent report of Tattersall and Pyke[23] indicates that the hereditary trait for human juvenile diabetes mellitus is not very strong, it seems reasonable to focus interest on environmental factors in the pathogenesis of this disease. N-nitrosomethylurea belongs to the N-nitroso compounds, which are present in the environment in low concentration and also may be formed endogenously[1-3]. A potential toxic effect of these compounds on the islet cells in man must therefore be considered.

SUMMARY

The uptake of labelled N-nitrosomethylurea in the pancreatic islets of mice and Chinese hamsters was studied. The substance was found to accumulate heavily in the islet tissue of the Chinese hamsters but not of mice. Pre-treatment with nicotinamide (500 mg/kg) seemed to have no effect on the uptake of the labelled substance in the

islets of the Chinese hamsters. Microautoradiographs showed an accumulation in both the β- and α-cells of the hamster islet tissue, mainly over the nuclei. The results indicated that the more specific diabetogenic effect of N-nitrosomethylurea in Chinese hamsters was caused by a selective uptake of this substance in the islet tissue, and that the ability of nicotinamide to block the diabetogenic activity of N-nitrosomethylurea in the same animal was not the result of inhibition of a specific uptake of this substance. The possibility that N-nitrosomethylurea may have a noxious effect on the islet tissue in man is considered.

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TABLE 1. Effect of intraperitoneally injected N-nitrosomethylurea on the blood glucose concentration in mice and Chinese hamsters after 2 days.

Treatment	Blood glucose (mg/100 ml) M ± SEM	
	MICE	CHINESE HAMSTERS
Buffer solution	132 ± 5 n = 5	96 ± 3 n = 16
N-nitrosomethylurea 50 mg/kg	138 ± 7[x] n = 5	159 ± 11[xx] n = 14
N-nitrosomethylurea 100 mg/kg	142 ± 6[x] n = 5	-
N-nitrosomethylurea 150 mg/kg	126 ± 20[x] n = 5	305 ± 75[xx] n = 5

[x] $p > 0.1$ in comparison with buffer injected mice

[xx] $p < 0.001$ in comparison with buffer injected Chinese hamsters

Fig. 1. Whole-body autoradiogram of a Chinese hamster 1 hour after an i.v. injection of ^{3}H-N-nitrosomethylurea, with an enlargement of the indicated pancreas. The pancreatic islets and the liver contain the largest amount of radioactivity (white areas) in the body.

g. 1 (original)

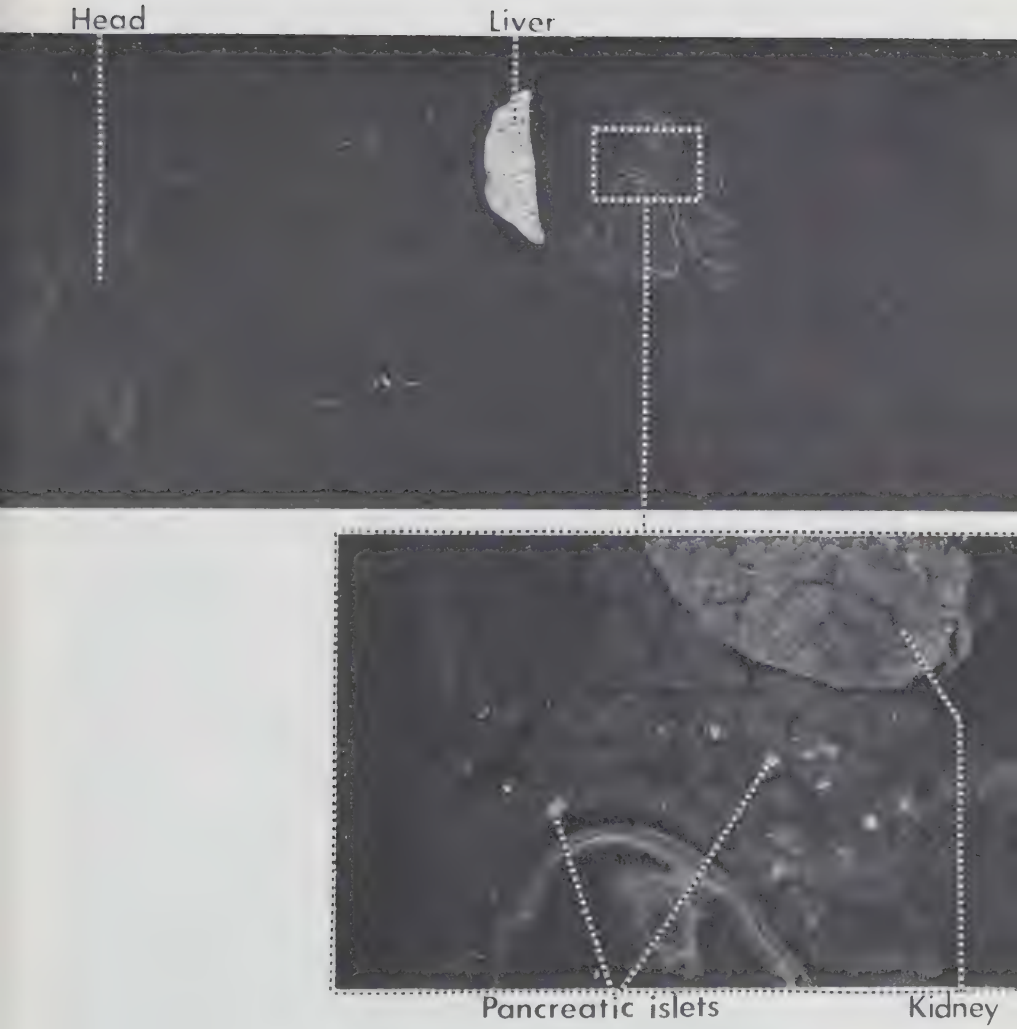


Fig. 2. Detail of a whole-body autoradiogram of a Chinese hamster 1 hour after an i.v. injection of ^{3}H-N-nitrosomethylurea: a) autoradiogram, b) hematoxylin-eosin stained section. High radioactivity is present in the pancreatic islets.

Fig. 2 (original)

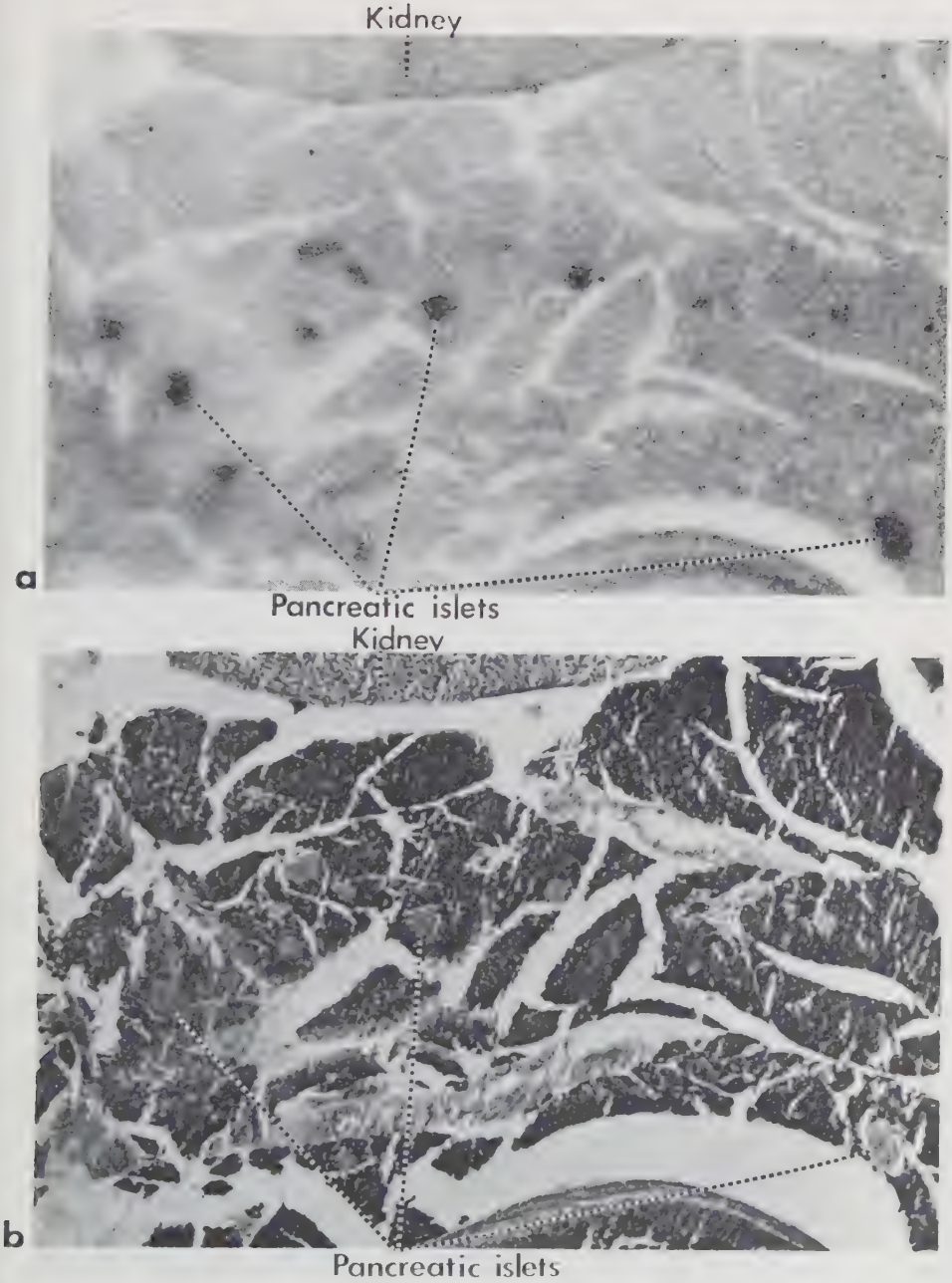


Fig. 3.a) Microautoradiogram of a [illegible] after i.v. injection of ^{3}H-N-nitrosomethylurea in a [illegible].

Fig. 3a (original)

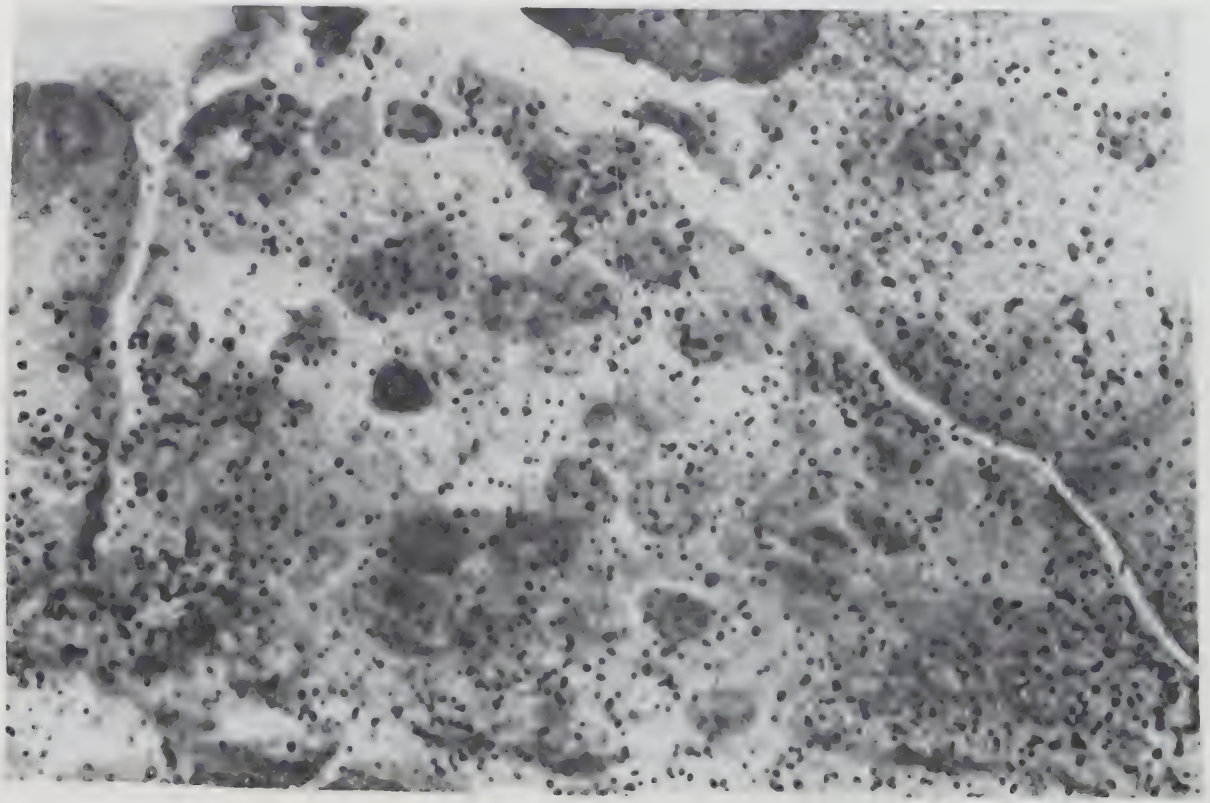


Fig. 3.b) The same islet after staining with aldehyde-fuchsin. The silver grains are found predominantly over the nuclei of both the β- and α-cells. Nuclear labelling is also seen in the exocrine pancreas. (X000).

Fig. 3b (original)

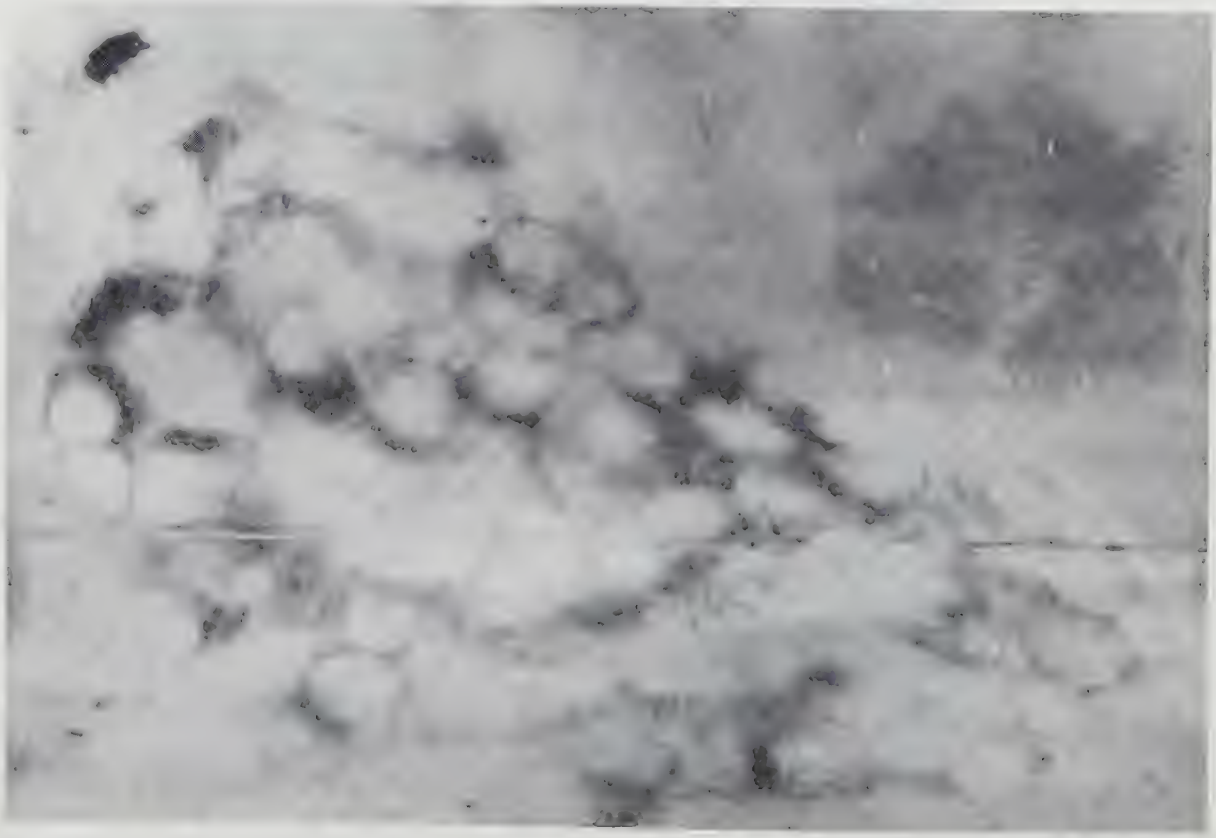


Fig. 4. Detail of a whole-body autoradiogram of a Chinese hamster 1 hour after an i.v. injection of ^{3}H-N-nitrosomethylurea. The animal was pre-treated with nicotinamide (500 mg/kg body weight) 10 minutes before the injection of ^{3}H-N-nitrosomethylurea: a) autoradiogram, b) hematoxylin-eosin stained section. Labelling of the pancreatic islets is observed also after pre-treatment with nicotinamide.

Fig. 4 (original)

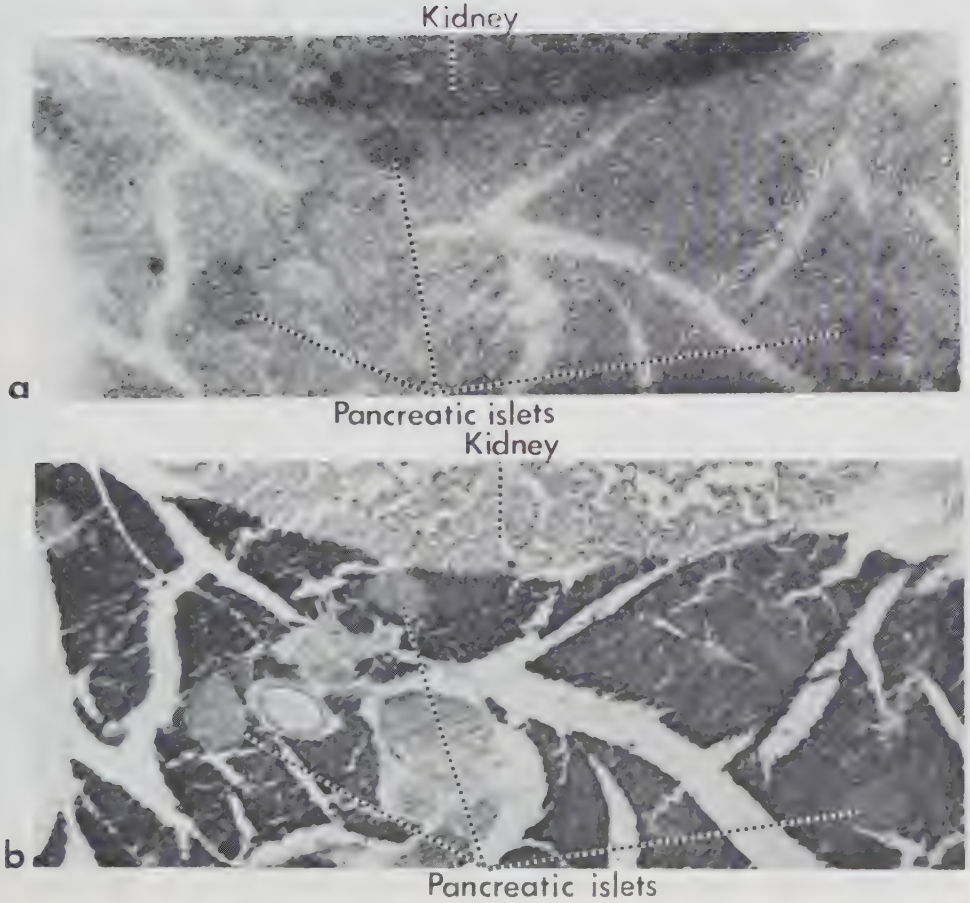
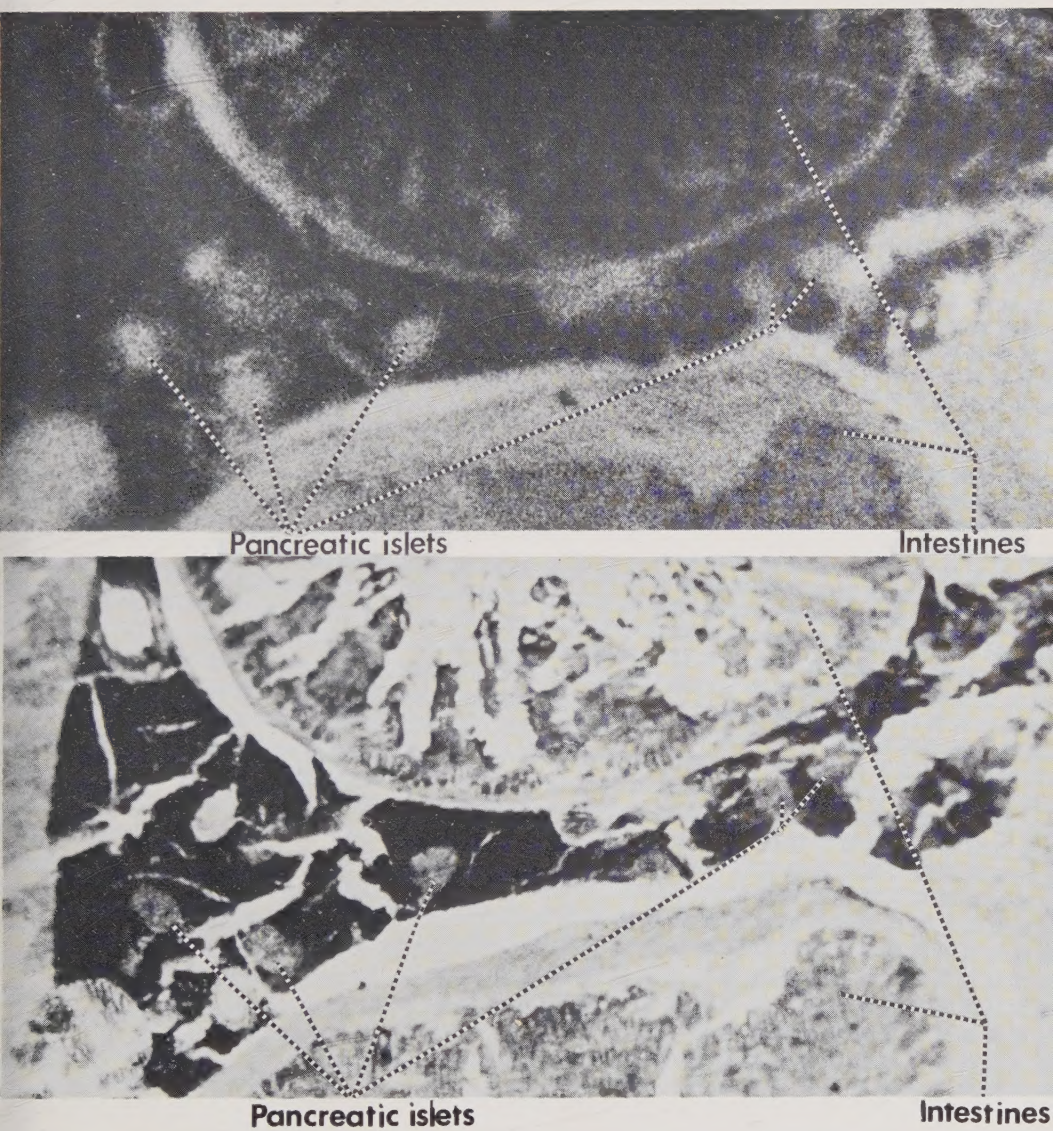


Fig. 5. Detail of a whole-body autoradiogram of an obese-hyperglycaemic mouse 1 hour after an i.v. injection of ^{3}H-N-nitrosomethylurea: a) autoradiogram, b) hematoxylin-eosin stained section. The radioactivity in the islets is low.

ig. 5 (original)



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